

# Petroleum HPV

201-14906

December 15, 2003

The Honorable Michael O. Leavitt, Administrator  
U.S. Environmental Protection Agency  
P.O. Box 1473  
Merrifield, VA 22116

**Attention: Chemical Right-to-Know  
HPV CONSORTIUM**

**Re: Reclaimed Substances Test Plan and Robust Summary**

Dear Administrator Leavitt:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Reclaimed Substances test plan and robust summary. The Petroleum HPV Testing Group is a consortium representing 92 percent of the nation's petroleum refining capacity. The Group is made up of 70 companies of the American Petroleum Institute (API), the National Petrochemical and Refiners Association (NPRA), the Gas Producers Association (GPA) and the Asphalt Institute (AI). Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and the associated test plans. We are therefore submitting this test plan, as well as the robust summary, directly to EPA to make available for public comment.

Note that the Testing Group has previously submitted a test plan entitled "Reclaimed Petroleum Hydrocarbons" that contained five chemical substances that were included in the original list of about 17 reclaimed substances submitted to EPA under the HPV Program.

For purposes of evaluating the remaining substances, the attached test plan created four chemical categories. Because the majority of the substances in these categories were intermediate streams or spent acids and caustics, it was the decision of the Testing Group that only one of the four categories, streams containing naphthenic acids, be evaluated for health and environmental effects. For that reason, there is only one robust summary included with the test plan.

Electronic copies of the test plan (in .pdf format) and robust summary (in .pdf format) are accompanying this letter via email to the EPA HPV robust summary email address (<http://www.epa.gov/chemrtk/srbstsum.htm>). An electronic copy of the robust summary as a IUCLID file also will be submitted at a later date. This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; [twerdokl@api.org](mailto:twerdokl@api.org)) or Tom Gray (202-682-8480; [grayt@api.org](mailto:grayt@api.org)) with any comments or questions you may have regarding this submission.

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Petroleum HPV Testing Group Oversight Committee and Technical Workgroup

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**HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM**

**TEST PLAN FOR RECLAIMED SUBSTANCES: STREAMS  
CONTAINING NAPHTHENIC ACIDS, PHENOLICS, DISULFIDES,  
ACIDS OR CAUSTICS**

**Submitted to the US EPA**

**by**

**The American Petroleum Institute  
Petroleum HPV Testing Group**

**[www.petroleumhvp.org](http://www.petroleumhvp.org)**

**Consortium Registration**

**December 15, 2003**

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## RECLAIMED SUBSTANCES TEST PLAN

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## **PLAIN LANGUAGE SUMMARY**

The high production volume (HPV) chemical substances addressed in this test plan are all intermediate streams or by-products that result from the refining of petroleum products. Most of these substances are not sold as products. Many are “spent” solutions that are sent to a chemical processor for recycling. For purposes of evaluating the sponsored substances, the Testing Group has created four categories: streams containing naphthenic acids, streams containing phenolics, streams containing disulfides, and streams containing acids or caustics. It is the Testing Group’s opinion that only one category, naphthenic acids, should be evaluated for the adequacy of health and environmental effects data.

Naphthenic acids are extracted from kerosene and diesel streams in the refinery to improve the burning qualities and storage properties of the finished products. The major uses of naphthenic acids are as oil-soluble metal soaps for driers and other catalysts, wood preservatives, tire cord adhesion promoters, and in amine derivatives for corrosion inhibitors. The available data show that naphthenic acids have a low to moderate degree of acute mammalian toxicity, and do not produce genetic toxicity in laboratory cell culture studies.

The Testing Group has determined that additional studies are necessary to adequately characterize the hazard of naphthenic acids. They are proposing that a combined repeated-dose, reproductive/developmental toxicity screening test be conducted and that genetic toxicity be evaluated in that study. In addition, toxicity testing in fish, aquatic invertebrates, and algae will be conducted to address the potential aquatic toxicity of these materials. There is adequate data on the physicochemical and environmental fate of naphthenic acids.

## **DESCRIPTION OF RECLAIMED SUBSTANCES CATEGORIES**

All of the sponsored HPV substances addressed by this test plan are intermediate streams or by-products from the refining of petroleum products. In addition, most of the substances are in some way associated with the use of caustic sodium hydroxide solutions to remove sulfur impurities from various process streams. Removal of sulfur impurities is important in petroleum refining to improve the burning qualities, storage stability, and odor of finished fuel products.

Caustic treatment also removes other constituents (some unintentionally) from the fuels including naphthenic acids and phenolics. Because of the low concentration of these impurities, it is not economical for refiners to recover and sell these chemical substances. However, spent caustics are routinely sent to a specialized chemical processor where some of them can be recovered and sold as products.

For purposes of evaluating the sponsored substances, the Testing Group has created four categories: streams containing naphthenic acids, streams containing phenolics, streams

containing disulfides, and streams containing acids or caustics. They are discussed individually below:

### **Streams Containing Naphthenic Acids**

Naphthenic acids are a naturally occurring, complex mixture of cycloaliphatic carboxylic acids recovered from petroleum distillates. The naphthenic acid containing chemical substances sponsored by the Testing Group are as follows:

#### **61790-13-4**

##### **Naphthenic acids, sodium salts**

#### **064754-89-8**

##### **Naphthenic acids (petroleum), crude**

A complex combination of compounds, predominantly naturally occurring organic acids, obtained from petroleum fractions by saponification and acidification. It consists predominantly of compounds which contain carboxylic acid functional groups and five- to six-member naphthenic rings in their molecular structures. Phenolic compounds and acidic sulfur compounds may also be present.

#### **1338-24-5**

##### **Naphthenic acids**

Naphthenic acids, as used in the petroleum industry, refers collectively to all of the carboxylic acids present in crude oil. Naphthenic acids (CASRN 1338-24-5) are classified as monobasic carboxylic acids of the general formula  $\text{RCOOH}$ , where R represents the naphthene moiety consisting of cyclopentane and cyclohexane derivatives. Naphthenic acids are composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids, with smaller amounts of acyclic aliphatic acids. The cycloaliphatic acids include single and fused multiple cyclopentane and cyclohexane rings. The carboxyl group is usually attached to a side chain rather than directly to the ring. Aromatic, olefinic, hydroxy and dibasic acids are present as minor components (Brient et al, 1995).

Although the presence of naphthenic acids has been established in almost all types of crude oil, only certain naphthenic and asphalt based crudes contain amounts that are high enough to require treatment in order to meet specifications. Naphthenic acids recovered from refinery streams occur naturally in the crude oil and are not formed during the refining process. Heavy crudes have the highest acid content, and paraffinic crudes usually have low acid content.

Naphthenic acids are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions. In addition to reducing corrosion in the refinery, the caustic wash of the distillates is necessary to improve the burning qualities, storage stability, and odor of the finished kerosene and diesel fuels. The commercial production of naphthenic acid from petroleum is based on the formation of sodium naphthenates when the petroleum distillates are treated with sodium hydroxide caustic. Since this reaction occurs *in situ*, naphthenic acids, sodium salts (CASRN 61790-13-4) is considered an intermediate stream in the production of naphthenic acid. The sodium

naphthenate-containing solutions contain approximately 5-15% sodium naphthenate, 0-0.5% sodium mercaptide and 3-4% sodium hydroxide in water, and the pH exceeds 12-13. These caustic solutions are typically sent to a specialized facility where they undergo further processing to recover the naphthenic acids.

The first step in recovery of the naphthenic acid involves “springing” (acidulating) the caustic solutions produced in the refinery to recover the organic acids. The resulting intermediate stream is crude naphthenic acids (petroleum), CASRN 64754-89-8. This is followed by a series of additional refining steps, including distillation, to recover the naphthenic acids.

The major uses of naphthenic acids are as oil-soluble metal soaps for driers and other catalysts, wood preservatives, tire cord adhesion promoters, and in amine derivatives for corrosion inhibitors. The petroleum industry uses naphthenic acid amine derivatives as surfactants for enhanced oil recovery and as corrosion inhibitors for refineries, pipelines, and downhole use.

**Summary** – of the three substances sponsored in this sub-category, naphthenic acids (CASRN 1338-24-5) is the only material sold commercially. The other two are intermediates in the production of naphthenic acids. Since all contain the same basic naphthenic acid species, information on the health and environmental effects of naphthenic acids can be extrapolated to the two intermediate streams. The Testing Group has reviewed the available health, environmental and physicochemical information on naphthenic acids and has used this material to represent the category.

### **Streams Containing Phenolics**

Cresols, xlenols and cresylic acids are produced during the catalytic cracking process and are concentrated in the middle distillate streams (approximately 350-700 degrees F) in the refinery. When any of these middle distillate streams are treated for mercaptan removal by caustic treating, cresols, xlenols and cresylic acids are co-extracted and become part of the caustic solutions. These caustic solutions are used as feedstocks to produce a number of finished products that are intermediates in the manufacture of a wide variety of industrial products such as resins, flame retardants, antioxidants, varnishes and disinfectants.

“Cresols” refer to any of the three isomers of methylphenol ( $C_7H_8O$ ) or combinations thereof. “Xlenols” are any of the six isomers of dimethylphenol ( $C_8H_{10}O$ ) and their various combinations. “Cresylic acid” is a generic term referring to combinations of both cresols and xlenols along with phenol or various other alkylphenols (ethylphenols, propylphenols, etc.).

The cresylate-containing chemical substances sponsored by the Testing Group are as follows:

### **68988-99-8**

**Phenols, sodium salts, mixed with sulphur comp; gasoline alky scrubber residues**

“A complex combination of phenolic compounds and sulfur compounds obtained from the treatment of gasoline with aqueous alkali at the catalytic cracking unit. It consists primarily of sodium salts of phenols, water neutral oils and sulfur compounds.”

This material is the spent caustic solution formed when catalytic cracked distillates are caustic treated to remove mercaptan sulfur compounds (the phenolic species, now in the form of sodium salts, i.e. cresylates, come along for the ride). Historically, this material has been the major feedstock at the facility where cresylic acid products are produced. This stream typically contains 10-15% sodium cresylates, 1% sodium mercaptide, 7.5% sodium hydroxide and 77-82% water, with a pH of 13.

#### **64743-03-9**

##### **Phenols, (petroleum)**

**Crude phenolic compounds (petroleum)** “A complex combination of organic compounds, predominantly phenol, cresols, xlenols and other alkylated phenols obtained primarily from cracked naphtha or distillate streams by alkaline extraction”

This material describes the intermediate product obtained when phenolic containing caustic solutions are acidified, thus “springing” cresylate compounds – converting them from a sodium salt to the organic form which then separates from the alkaline material. Further processing of this “sprung” organic mixture is necessary to separate the individual chemical compounds from the mixture. This process is not commonly carried out in refineries; rather the caustic solutions are sent to a specialized facility for conversion of the cresylates and recovery of the phenolic products. This intermediate stream typically contains about 20% phenol, 40% cresols (o-, m-, and p-isomers), 30% xlenols (6 isomers) and 10% alkylated phenols.

**Summary** - It is the HPV Testing Group’s conclusion that none of the materials in this subcategory should be evaluated in the HPV program. The first stream (68988-99-8) is a spent caustic and should not be used in animal studies for obvious reasons. Because it is a byproduct, and processed only by a few companies, its potential for human and environmental exposure is very limited.

The second stream (64743-03-9) is also one in which there is limited potential for human or environmental exposure since it is a non-isolated intermediate in the process used to recover cresylic acid components. As a non-isolated intermediate, its reporting on the Inventory Update Report was probably in error. That being the case, the stream would not be considered a “manufactured” chemical substance and not eligible for inclusion in the HPV program. In addition, EPA’s Tier 2 gasoline requirements will result in most refiners replacing caustic extraction with more efficient sulfur removal technologies.

**It should also be noted that phenols and cresols have been the subject of American Chemistry Council Chemstar panels. While the testing group did not evaluate these chemicals for data adequacy, it is believed that there is substantial information on their potential health and environmental hazards.**

## **Streams Containing Disulfides**

Petroleum crude oils contain organic sulfur compounds (mercaptans), dissolved hydrogen sulfide and sometimes suspended sulfur. High concentrations of sulfur compounds are undesirable in petroleum refining since they can be corrosive, reduce the service life of certain catalysts, and degrade the quality of finished products by changing their color or by giving them an unpleasant odor. High sulfur also increases the formation of sulfates and sulfites during the combustion of petroleum fuels. Mercaptans have the general formula  $R - S - H$ , where R represents an aliphatic or cyclic radical.

Removing mercaptans from various streams is referred to as sweetening and is used most commonly for gas, naphtha and kerosene range streams. Sweetening can be effected in one of two modes, extractive and non-extractive. In the extractive mode, mercaptans are caustic extracted from the hydrocarbon stream and later oxidized to disulfides. In the non-extractive mode the caustic treatment is carried out in the additional presence of oxygen (air) and the mercaptides are oxidized *in situ* to disulfides which are reverse extracted back into the hydrocarbon stream. In the extractive version, sodium hydroxide (caustic) treatment of the gas or naphtha streams will remove the mercaptan sulfur compounds as mercaptides in the caustic solution and in a separate process step the mercaptides in the caustic solution are oxidized to disulfides.

Disulfides have the general formula  $R - S - S - R$ , and are not corrosive. The disulfides are typically removed from the caustic by solvent extraction. They are not typically separated by refiners for commercial sale because the small volumes produced are not economical for commercial production. The solvent solutions containing the disulfides are typically recycled in the refinery upstream of a hydrotreater which results in destruction of the disulfide substances. They may also be burned as a refinery fuel. In addition, there are only limited uses for these materials. The primary outlet for disulfides is the manufacture of sulfuric acid where they are burned for sulfur recovery. They have also been used as rubber reclaim solvents and as downhole desulfurization solvents in the oil patch.

As described above, EPA's requirements for reducing the sulfur level in fuels has resulted in an increase in the use of hydrotreating and other sulfur removal technologies, since caustic treatment is not as efficient. This has decreased the amount of spent caustic being generated. Merichem is currently the only company that still handles phenolic containing caustics as feedstocks in its plant to make cresylic acid products. As a result of the decrease in feedstock availability, they will be changing their process in 2005 and no longer manufacturing disulfide oils.

The disulfide-containing chemical substances sponsored by the Testing Group are as follows:

**68334-01-0**

**Disulfides, alkylaryl dialkyl, petroleum refinery spent caustic oxidation products**

“A complex combination of disulfides obtained from oxidation of spent caustic from the caustic treatment of straight run naphtha and catalytic cracked naphtha.”

This material, if produced by a refiner, represents the product obtained by oxidizing the spent caustic used in removing mercaptans from straight run and catalytic cracked naphtha streams. It is typically recycled on site or burned as a fuel. It is a hydrocarbon material and not corrosive. As mentioned above, disulfide oils are often produced in admixture with a solvent and if recycled in the refinery, is typically inserted upstream of a hydrotreater or other process that removes the sulfur. It is not sold as a product.

**68955-96-4**

**Disulfides, dialkyl and di-Ph, naphtha sweetening**

"A complex combination of disulfides obtained by subjecting naphtha or gases from various refinery processes to a sweetening process to convert mercaptans. The dialkyl disulfides have carbon numbers predominantly in the range of C1 through C4.”

This material represents the mixture of disulfides that results from caustic extraction of naphtha streams, followed by oxidation of the resulting caustic solution containing the extracted mercaptides. In refineries, the disulfides are either recycled back into the refinery or burned as fuel. It is not sold as a product.

**68513-62-2**

**Disulfides, C5-12-alkyl**

This material represents the disulfides resulting from sweetening of heavy straight run or cracked naphtha streams. Only one refiner in the HPV testing group reported this material. They indicated that the material is completely recycled back into the refinery and not sold as a commercial product.

**68920-64-9**

**Disulfides, di-C1-2-alkyl**

This CASRN refers to the disulfides obtained from sweetening a light hydrocarbon gas or LPG type streams. The mercaptans in the stream are removed by caustic extraction and thereafter the caustic solution is oxidized with air to form the disulfides, which may be facilitated in their separation by the use of a solvent. The resulting disulfide/solvent mixture is either recycled within the refinery or burned as a fuel. This occurs in a closed system. This material is not sold by refiners as a commercial product.

**Summary** – All four materials described in this subcategory are mixtures of disulfides (organic materials) represented by the formula R-S-S-R. All four, if produced in the refinery, are either recycled within the refinery or burned as a fuel. None of these materials are sold as a product of the refinery. The disulfides produced by Merichem, the only company that reclaims them from spent caustics, are subsequently destroyed in the manufacture of sulfuric acid. Their manufacture of disulfides will cease in 2005.

It is the HPV Testing Group's conclusion that none of the materials in this subcategory should be evaluated in the HPV program.

### **Streams Containing Acids or Caustics**

This subcategory contains materials that are either highly acidic or highly alkaline. These materials are all byproducts of petroleum processing and are not sold as consumer products.

#### **68815-21-4**

##### **Tar acids, cresylic, sodium salts, caustic solutions.**

This material is a spent caustic solution obtained from the neutralization of acidic petroleum distillate streams. Its composition is predominantly sodium salts of cresylic and phenolic acids, sodium hydroxide and water. This material is either sent to a chemical processor for recovery of organic acids or disposed of as a waste. It is highly alkaline and corrosive.

#### **064742-24-1**

##### **Sludges (petroleum), acid**

A complex combination of sulfuric and sulfonic acids, water, esters and high molecular weight organic compounds such as polymers of olefinic hydrocarbons. It is formed during the treating of petroleum fractions with sulfuric acid.

This material was only reported by one refiner. Sulfuric acid is used as a catalyst in the alkylation process. While much of the acid is recycled back through the acid plant for reuse, a stream of contaminated acid is removed to reduce the buildup of impurities. The spent acid stream is used only for pH control at the refinery's wastewater plant and none is shipped off-site. The sulfuric acid concentration of this material is between 90-93%.

#### **064742-40-1**

##### **Neutralizing agents (petroleum), spent sodium hydroxide**

A complex combination consisting predominantly of water and containing sodium hydroxide and organic and inorganic sodium salts.

This material, as described above, is produced by treating certain hydrocarbon streams for mercaptan removal. Some of the caustics come from the caustic treating units while others come from regeneration units where the caustics are initially oxidized to remove the extracted sulfur compounds as disulfides. These units return most of the caustic to the treating unit, but there is a draw down required to keep the sulfides from concentrating to the point where the regenerated caustic is no longer useful. The typical concentration of the spent caustic is approximately 4 % sodium sulfide, 5 % sodium hydroxide, 1% sodium mercaptides, and 90% water.

**Summary** – All of these materials are highly alkaline or acidic by-products of various petroleum refining processes. It is the Testing Group's conclusion that none of the materials in this subcategory should be evaluated in the HPV program.

### **CATEGORY RATIONALE**

Because the majority of the substances in these categories were intermediate streams or spent acids and caustics, it was the decision of the Testing Group that only one of the four categories, streams containing naphthenic acids, be evaluated for health and environmental effects. Of the three substances sponsored in this subcategory, naphthenic acids (CASRN 1338-24-5) is the only material sold commercially. The other two are intermediates in the production of naphthenic acids. Since all contain the same basic naphthenic acid species, information on the health and environmental effects of naphthenic acids can be extrapolated to the two intermediate streams. The Testing Group has reviewed the available health, environmental and physicochemical information on naphthenic acids and has used this material to represent the category.

### **EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING**

For the reasons stated above, the only reclaimed substances subcategory being evaluated for existing health effects data is naphthenic acids. This section addresses the mammalian toxicity endpoints by:

1. Searching the literature for existing data and evaluating existing studies for adequacy;
2. Using read-across information whenever possible of similar materials; and
3. Proposing toxicity testing needed, when necessary to fill data gaps.

The endpoints include: acute toxicity, repeated dose toxicity, *in vitro* and *in vivo* mutagenicity, and reproductive/developmental toxicity. Where complete studies were not available for review (e.g., technical meeting abstracts or poster presentations), these data are summarized here and the source citations are provided in the Reference section of this document. In addition, information from relevant non-OECD SIDS/HPV Chemical Program protocols is cited and judged for its applicability to the Test Plan.

### **Streams Containing Naphthenic Acids**

#### **Acute Toxicity**

The acute toxicity of naphthenic acids has been investigated in experimental animals via the oral and dermal routes of exposure. In male rats, the acute oral LD<sub>50</sub> of a naphthenic acids sample was determined to be 5.88 g/kg body weight (Exxon, 1979b). Although no deaths were observed in the low dose animals (1.0 and 2.15 g/kg), a number of symptoms of toxicity were noted. In a previous acute oral screening LD<sub>50</sub> study, both female and male rats were administered 10 g/kg, after which all the animals died (Exxon, 1979b). In rabbits, the same material had a dermal LD<sub>50</sub> of >3.16 g/kg (Exxon, 1979c). Toxic signs were observed and the skin reactions were judged to be moderately to severely irritating (Exxon, 1980b). This material was also determined to be moderately irritating to the eyes of rabbits (Exxon, 1979d; Exxon, 1980b). An inhalation study in rats, mice and guinea pigs at doses of 0.63 mg/l (near saturation level) for 6 hours was reported to

produce no deaths or signs of systemic toxicity (Exxon, 1987); however, experimental details of this study could not be verified.

Oral LD<sub>50</sub>s of 3.0 g/kg and 5.2 g/kg were observed in rats dosed with a naphthenic acids fraction from crude kerosene acids or mixed crude acids respectively (Rockhold, 1955). In another study, the oral LD<sub>50</sub> of naphthenic acids was determined to be 3550 mg/kg in young white male mice (Pennisi and Lynch, 1977 -abstract only). The quality of this study cannot be verified since the report does not provide complete experimental details.

In a study using a non-OECD SIDS/HPV Chemical Program protocol, adult female rats received a single oral dose of naphthenic acids of 3, 30, or 300 mg/kg body weight while adult male rats received 300 mg/kg. After 14 days, treatment-related effects on the cardiovascular system and liver, in particular, were observed in the high dose groups of both sexes (Rogers et al, 2002a). In addition, significant cerebral hemorrhages were observed in male rats.

**Summary: No additional testing is planned.** Available data suggest a low order of acute toxicity, with significant systemic toxicity at doses that are not lethal. The Testing Group has determined that the data are of sufficient quality to estimate the acute toxicity potential of naphthenic acids.

#### **Repeat-Dose Toxicity**

A 90-day oral gavage study (non-OECD SIDS/HPV Chemical Program protocol) in female rats of naphthenic acids at doses of 0.6, 6, and 60 mg/kg/day resulted in a number of apparent treatment-related effects (Rogers, et al, 2002a). These include the following: body weight decreases, increases in relative liver, brain and kidney weights, plasma biochemical differences indicating the liver as a target organ, and increased glycogen storage in the liver. All effects occurred in the high dose groups. In addition, there was a severe seizure activity observed in high and mid-dose animals after day 40. The authors noted that there were milder episodes observed in the low-dose and control groups as well. The limited number of organs examined and the absence of male data limit the usefulness of this study in estimating repeat-dose toxicity.

Male mice administered an oral dose of naphthenic acid at 1000 mg/kg/day for 30 days demonstrated signs of CNS depression, hematological changes, weight loss (leading eventually to death due to respiratory arrest), gross morphological changes in the liver and stomach, and histomorphological changes in a few unidentified, selected organs (Pennisi and Lynch, 1977 -abstract only). The quality of this study cannot be verified since the report does not provide complete experimental details.

In a two-year dermal carcinogenicity study in female mice (no male data), calcium naphthenate (the calcium salt of naphthenic acids) in mineral oil (unspecified concentration) resulted in a carcinogenic response after 392 days of treatment. No metastatic tumors were present. (U.S.EPA, 2003 - Shell, 1982). Only brief reports of this study are available, consequently the quality of the study cannot be evaluated.

**Summary:** The Testing Group thinks the existing repeat-dose toxicity studies on naphthenic acids are not of sufficient quality to adequately address this endpoint. Consequently, naphthenic acid will be tested (route to be determined) using a 28-day combined, repeated-dose and reproductive/developmental toxicity screening protocol (OECD Test Guideline 422). It is the intention of the Testing Group to augment the 28-day dermal study with the *in vivo* micronuclei assay to provide additional information on the genotoxicity of naphthenic acid (See “In Vivo Mutagenicity”).

#### **In Vitro Mutagenicity**

Although no studies were available on the *in vitro* genotoxicity of naphthenic acid, there are data on the calcium and sodium salts. NTP studies indicate that neither calcium naphthenate nor sodium naphthenate were mutagenic in *S. typhimurium* with or without S9 (NTP, 2003). Sodium naphthenate did not produce effects in hamster ovary cells, but was positive in a sister chromatid exchange assay. Calcium naphthenate was not mutagenic in *Escherichia coli* or yeast (*Saccharomyces cerevisiae*), and did not cause chromosome damage in rat liver cells (BIBRA, 1999). The details of these calcium naphthenate studies could not be verified.

**Summary: No additional testing is planned.** Available NTP data on the sodium and calcium salts of naphthenic acid suggest a low potential for genotoxicity. The Testing Group has determined that the data are of sufficient quality to estimate the *in vitro* genotoxic potential of naphthenic acids.

#### **In Vivo Mutagenicity**

No studies have been reported on the *in vivo* genotoxicity of naphthenic acids or related materials.

While the Testing Group shares the desire to limit animal testing by using *in vitro* methodologies when possible, it decided to conduct the *in vivo* micronucleus test because it could be performed using animals that were already included in the repeat dose 28-day study, and it eliminates the need to perform an additional study solely for the purpose of studying *in vivo* genotoxicity.

**Summary:** Naphthenic acid will be tested in the *in vivo* mammalian erythrocyte micronucleus test (OECD 474). The *in vivo* micronucleus test will be included in the 28-day repeat dose study on naphthenic acid (see “Repeat-Dose Toxicity” section).

#### **Reproductive/Developmental Toxicity**

No studies have been reported on the reproductive toxicity of naphthenic acids. In a non-OECD SIDS/HPV Chemical Program protocol, neat calcium naphthenate solution (concentration not provided) was applied to male rabbits (10 total), which were subsequently mated to untreated females (2 total). No significant effects were seen in reproductive parameters or abnormalities in the male reproductive tract. The description and results of this study are in abstract form only (U.S. EPA, 2003 – website).

Naphthenic acids extracted from tar sands have been reported to affect female fertility in rats exposed to oral doses of 60 mg/kg/day during pre-breeding, breeding, and gestation (93% success in controls vs. 7% treated). In addition, total cholesterol of the treated group was 30% lower than controls. No fetal malformations were reported (Rogers et al, 2002b –abstract only). The quality of this study cannot be verified since the report does not provide a complete set of experimental details.

**Summary:** The Testing Group does not think that the data are of sufficient quality to estimate the reproductive or developmental toxicity of naphthenic acids. Naphthenic acids will be tested using a 28-day combined, repeated dose and reproductive/developmental toxicity screening protocol (OECD Test Guideline 422) (See “Repeated Dose Toxicity”).

## **EVALUATION OF EXISTING PHYSICOCHEMICAL AND ENVIRONMENTAL FATE DATA**

### **Streams Containing Naphthenic Acids**

#### **Physicochemical Data**

Although some data for products in this subcategory exist, not all of the physicochemical SIDS endpoints are defined and a consensus database for chemicals that represent products in this subcategory does not exist. Therefore, calculated and measured representative data have been identified and a technical discussion provided, where appropriate. The EPIWIN<sup>®</sup> computer model (U.S. EPA, 2000), as discussed in the US EPA document entitled "*The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program*" (U.S. EPA, 1999a) has been used to calculate physical-chemical properties of representative constituents of naphthenic acids.

Because of the diversity of compounds encompassing naphthenic acids, it is not feasible to model the physicochemical endpoints for each potential compound. Modeling efforts were directed towards those constituents of naphthenic acids covering representative molecular weights and isomeric structures most likely to exist in the streams defined in this category.

#### **Melting Point**

Because naphthenic acids are not pure chemicals, the melting point characteristics of these complex mixtures vary with the hydrocarbon composition of their make-up. A technical discussion of factors affecting the melting point characteristics provided in the robust summary used information from published sources and modeled values using EPIWIN, MPBPWIN Version 1.40 (U.S.EPA, 2000). Based on data available in commercial product specifications and Material Safety Data Sheets (MSDS), substances produced for commercial use have melting points that fall in the range from –35 °C to +2 °C. Estimated melting points for constituent cycloalkyl carboxylic acids representative of molecular weight and carbon numbered naphthenic acids extracted from petroleum streams ranged from 117 to 160 °C. Actual melting ranges will vary depending upon the constituent hydrocarbons in the naphthenic acid mixture. Additional steps used in the

refining of the commercial products may explain differences among melting points of those products and waste streams.

**Summary: No additional testing is proposed.** Adequate data are presented to characterize the melting points of naphthenic acid mixtures.

### **Boiling Point**

Because these substances are not pure chemicals, the boiling point characteristics of naphthenic acids and their salts vary according to the hydrocarbon component make-up of the complex mixture. Therefore, a technical discussion of factors affecting the distillation range characteristics was provided in the robust summary using information from published sources and EPIWIN, MPBPWIN Version 1.40 (U.S.EPA, 2000). Based on data available in commercial product specifications and Material Safety Data Sheets (MSDS), substances produced for commercial use have boiling points that fall in the range from 140 °C to 370 °C. Estimated boiling points for constituent cycloalkyl carboxylic acids representative of molecular weight and carbon numbered naphthenic acids extracted from petroleum waste streams ranged from 233 to 375 °C. Actual boiling ranges will vary depending upon the constituent hydrocarbons in the naphthenic acid mixture. Additional steps used in the refining of the commercial products may explain differences in the boiling ranges of those products and waste streams.

**Summary: No additional testing is proposed.** Adequate data are presented for distillation ranges for naphthenic acids.

### **Vapor Pressure**

Commercial product data typically provided narrative comments such as “negligible”, “very low”, or “not applicable” for vapor pressures of those substances (SocTech 2003; AGS Chemicals Limited 2003; Mallinckrodt Baker Inc. 1997). Because naphthenic acids are complex mixtures, the vapor pressure of the mixture is a function of the sum of the vapor pressures of the components in their pure state times their mole fraction in the mixture (Raoult’s Law). Estimates of the vapor pressures of constituent naphthenic acid compounds reported by Rogers et al. (2002c) to predominate in extracts of oil sands tailings water were made using EPIWIN (U.S. EPA 2000). A technical discussion of those estimates and predicted vapor pressures for naphthenic acid mixtures was developed in the robust summary. Based upon modeled estimates of representative constituent naphthenic acid structures, vapor pressures ranged from  $1.8 \times 10^{-3}$  to  $1.4 \times 10^{-5}$  Pa.

**Summary: No additional testing or modeling is proposed.** Adequate characterization of vapor pressure for naphthenic acids was developed.

### **Partition Coefficient**

Due to their complex composition, unequivocal determination of the log  $K_{ow}$  of naphthenic acid mixtures cannot be made. To gain an understanding of the partitioning potential of these substances, partition coefficients of selected molecular weights and naphthenic ring structures were modeled using EPIWIN<sup>®</sup> (U.S. EPA, 2000). Structures

were selected that have been reported to represent the predominant range of molecular weights and ring constituents of naphthenic acids found in crude oil extracts (Rogers et al. 2002c). Estimates demonstrated that molecules containing longer carboxyl side chains tend to have higher partition coefficients. Also, multi-ring compounds tend to have lower partition coefficients than single-ring compounds of equal molecular weight. Partition coefficients for the modeled naphthenic acid structures ranged from 5 to >6. This range of values is based on structures known to predominate in some naphthenic acid extracts; however, lower partition coefficients would be predicted for structures having lower molecular weights.

**Summary: No additional testing or modeling is proposed.** Partition coefficients of selected molecular weight naphthenic acids commonly found in complex mixtures were estimated.

### **Water Solubility**

Because naphthenic acids exist as mixtures of many different compounds having a variety of molecular weights, number of cycloalkane rings, and alkyl side chains, the water solubility of substances in this subcategory will depend upon the compositional make-up of the complex mixture (Havre, 2002). Also affecting solubility is the pH of the naphthenic acid solutions. Naphthenic acids are weak acids having pKa values of approximately 5 (CEATAG, 1998; Havre, 2002). The greatest proportion of the total acid in solutions having pHs >5 would exist in the ionic form, while solutions having pHs <5 would exist mostly in the molecular form (Havre, 2002). Therefore, alkaline solutions increase a naphthenic acid's solubility, and acid solutions decrease solubility (Havre 2002).

Data cited in commercial product literature vary widely and suggest that degree of refining in order to meet product performance specifications may greatly influence the solubility of the end product. These product literature reports are largely unassignable with respect to reliability, but they have cited solubility values as high as 5000 mg/l at pH 9 (CEATAG, 1998). In contrast, other product literature references have cited narrative statements such as "very low water solubility" (SocTech S.A., 2003), or "only slightly soluble in water" (AGS Chemicals Limited, 2003).

Because naphthenic acid mixtures may contain hundreds to thousands of individual compounds, the EPIWIN<sup>®</sup> computer model was used to estimate a range of water solubility values for selected naphthenic acid structures reported by Rogers et al. (2002c) to predominate in some natural naphthenic acid extracts. A technical discussion was prepared in the robust summary to describe factors affecting the water solubility of these substances. Estimated solubility values ranged from 0.0003 to 2.1 mg/l depending upon the molecular weight.

**Summary: No additional testing or modeling is proposed.** Water solubility values of selected molecular weight naphthenic acids commonly found in complex mixtures were presented.

### **Photodegradation**

Atmospheric oxidation as a result of hydroxyl radical attack is indirect photodegradation. Substances in this subcategory have low vapor pressures and therefore do not have a tendency to volatilize to air where they can undergo reactions with photosensitized oxygen in the form of hydroxyl radicals ( $\text{OH}^\cdot$ ). Therefore, these reactions are not expected to be an important environmental fate process.

The potential to undergo indirect photodegradation was estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN<sup>®</sup> (U.S.EPA, 2000), which calculates a chemical half-life and an overall  $\text{OH}^\cdot$  reaction rate constant based on a 12-hour day and a given  $\text{OH}^\cdot$  concentration. Atmospheric oxidation rates and half-lives were thus calculated for a range of molecular weight and naphthenic ring structures covering one to four-ring cycloalkyl carboxylic acids having molecular weights from 254 to 325. These structures were considered appropriate because they have been found to predominate in naphthenic acid extracts from Athabasca oil sands, a source considered high in naphthenic acid content (Rogers et al., 2002c).

AOP half-life estimates for these compounds ranged from 0.3 to 0.6 days and show a lack of persistence in the atmosphere. However, with vapor pressures of  $1.8 \times 10^{-3}$  to  $1.4 \times 10^{-5}$  Pa, there is low potential for these substances to partition to the atmosphere where indirect photodegradation would occur.

**Summary: No additional modeling is proposed.** The atmospheric oxidation potential of representative components in naphthenic acids has been estimated.

### **Stability in Water**

Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). Because naphthenic acids do not contain significant levels of these functional groups, components in the naphthenic acid subcategory are not subject to hydrolysis.

**Summary: No additional modeling is proposed.** Components in the naphthenic acids subcategory do not undergo hydrolysis.

### **Chemical Transport and Distribution (Fugacity Modeling)**

Fugacity-based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (e.g., air, water, soil, sediment, suspended sediment and biota). The U.S.EPA has agreed that computer modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model (Mackay 1991). The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. EPA cites

the use of this model in its document “Determining the Adequacy of Existing Data” that was prepared as guidance for the HPV chemicals program (U.S. EPA, 1999b).

To gain an understanding of the potential transport and distribution of naphthenic acids, the EQC model was used to characterize the environmental distribution of different molecular weights and structural conformations of naphthenic acid molecules. These constituents were selected because they had been shown to predominate in extracts of Athabasca oil sands (Rogers et al., 2002c). Modeling results show that when naphthenic acids are released to the environment, they would bind to soil/sediments, with negligible fractions partitioning to air, water or biota. However, not all naphthenic acids are composed of identical chemical species as reported by Rogers et al. (2002c), and mixtures having a predominance of low molecular weight constituents would be expected to partition to some degree to water depending on their pKa characteristics and the pH of the water.

**Summary: No further modeling is proposed.** Fugacity modeling has been done to provide an estimate of the percent distribution in environmental media of various molecular weight and ring-structured naphthenic acids.

### **Biodegradation**

Although no standardized ready or inherent biodegradation studies were available for naphthenic acids, research has shown these materials to be amenable to microbial utilization similar to other hydrocarbon compounds. Studies have demonstrated that microorganisms indigenous to oil sands tailings were capable of degrading complex mixtures of commercial sodium salts of naphthenic acids as well as mixtures of organic acids extracted from oil sands tailings (Herman et al., 1993, 1994). Although rates of biodegradation may be affected by steric factors related to the numbers of cycloalkane rings or the alkyl constituents on the ring structure, microbial populations respond to naphthenic acid substrates through increased CO<sub>2</sub> production, O<sub>2</sub> consumption, and enhancement of metabolism with the addition of nutrients. With single-ring naphthenic acids, biodegradation of both the ring and side-chain acid has been shown to occur (Herman et al., 1993, 1994). As the number of cycloalkane rings increase, it may be inferred from what is known about degradation of multi-ring naphthenes that biodegradation rates may slow, but these substances will degrade given time (Bartha and Atlas 1977). Biodegradation (as percent of organic carbon converted to CO<sub>2</sub>) of model naphthenic acid compounds, cyclohexane carboxylic acid, cyclohexane pentanoic acid, 2-methyl cyclohexane carboxylic acid, and *trans*-4-pentylcyclohexane carboxylic acid ranged from 6 to 67% depending on the micro-organism culture and the presence of nitrogen and phosphorus nutrients in the medium (Herman et al., 1994).

**Summary: No further testing is proposed.** An adequate characterization of the potential for naphthenic acid biodegradation to occur has been made. A technical review of current research on microbial utilization of naphthenic acids has been included in the robust summary.

## EVALUATION OF EXISTING ECOTOXICITY DATA AND PROPOSED TESTING

### Streams Containing Naphthenic Acid

Aquatic toxicity endpoints for the OECD SIDS/HPV Chemical Program include acute toxicity to a freshwater fish and invertebrate, and toxicity to a freshwater alga. There are no standard guideline studies on the toxicity of naphthenic acids to these aquatic organisms. Toxicity endpoints reported in the literature largely report nominal lethal concentrations with varying amounts of supporting information on test conditions. The available data show that naphthenic acids are moderately to highly toxic to fish. Cairns et al. (1965) reported the 96-hour TLM of 16.3 mg/l for adult zebra fish (*Brachydanio rerio*) and the 48-hour TLM of 3.5 mg/l for embryos based on nominal concentrations. Dorn (1992) found the 96-hour LC50 for three-spine sticklebacks (*Gasterosteus aculeatus*) to be between 2.5 and 5 mg/l also based on nominal concentrations. Other data not considered to provide sufficient detail for assessment included a report of a 48-hour TLM for bluegill (*Lepomis macrochirus*) of 5.6 mg/l (Cairns and Scheier, 1962) and a report of a 96-hour LC50 for bluegill of 0.0026 mg/l (Exxon 1980a).

No studies were found that measured acute toxicity to freshwater invertebrates or algae.

**Summary:** The Testing Group intends to conduct the following studies on naphthenic acids: OECD Guideline 203, Fish Acute Toxicity Test; Guideline 202, Daphnia sp., Acute Immobilization Test; and Guideline 201, Alga Growth Inhibition Test.

## MATRIX OF AVAILABLE ADEQUATE DATA AND PROPOSED TESTING

**TABLE 1: Naphthenic Acid: Matrix of Available Data and Proposed Testing**

TEST	DATA ASSESSMENT
<b>Physical/Chemical Properties</b>	
Melting Point	Adequate
Boiling Point	Adequate
Vapor Pressure	Adequate
Partition Coefficient	Adequate
Water Solubility	Adequate
<b>Environmental Fate</b>	
Photodegradation	Adequate
Stability in Water	Adequate
Chemical Transport and Distribution (Fugacity Modeling)	Adequate
Biodegradation	Adequate
<b>Ecotoxicity</b>	
Algae Growth Inhibition	TEST
Acute Feshwater Invertebrate	TEST
Acute Freswater Fish	TEST
<b>Mammalian Toxicity</b>	
Acute	Adequate
Repeat Dose	TEST
Genetic Toxicity, <i>In vitro</i>	Adequate
Genetic Toxicity, <i>In vivo</i>	TEST
Repro/Development	TEST

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201-14906B

**ROBUST SUMMARY  
OF INFORMATION ON**

**Substance Group: RECLAIMED SUBSTANCES:  
NAPHTHENIC ACID**

RECEIVED  
OPT CBIC  
03 DEC 17 PM 2:39

**Summary prepared by: American Petroleum Institute**

**Date of last Update: DECEMBER 15, 2003**

**Number of pages: 40**

Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H.J., Andreae, M. and Tillman, U (1997)

A systemic approach for evaluating the quality of experimental toxicological and exotoxicological data.  
Regulatory Toxicology and Pharmacology 25: 1-5

## 1. General Information

### 1.1 GENERAL SUBSTANCE INFORMATION

**Substance Type:**

Naphthenic Acids

**Physical status:**

Naphthenic acid fractions are oily liquids. The salts may be liquid or solid. Naphthenic acids (CASRN 1338-24-5) are classified as monobasic carboxylic acids of the general formula  $\text{RCOOH}$ , where R represents the naphthene moiety consisting of cyclopentane and cyclohexane derivatives. Naphthenic acids are composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids, with smaller amounts of acyclic aliphatic acids. The cycloaliphatic acids include single and fused multiple cyclopentane and cyclohexane rings. The carboxyl group is usually attached to a side chain rather than directly to the ring. Aromatic, olefinic, hydroxy and dibasic acids are present as minor components.

Naphthenic acids recovered from refinery streams occur naturally in the crude oil and are not formed during the refining process. Heavy crudes have the highest acid content, and paraffinic crudes usually have low acid content. Naphthenic acids are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions.

## 2. Physical and Chemical Data

### 2.1 MELTING POINT

<b>Test Substance:</b>	Naphthenic Acids, commercial mixtures		
<b>Method:</b>	Not stated		
<b>Year (Guideline):</b>	Not stated		
<b>Type (test type):</b>	Not stated		
<b>GLP:</b>	Unknown		
<b>Test Conditions:</b>	Unknown		
<b>Results:</b>	-35 °C to +0 °C	Ref (1)	
	-35 °C to +2 °C	Ref (2)	
	+30 °C	Ref (3)	
<b>Remark:</b>	Values cited represent ranges of melting points cited in product literature data and Material Safety Data Sheet for commercial naphthenic acid products.		
<b>Source:</b>	(1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: <a href="http://www.soctech.ro/English/Produse/1acizinaft.htm">http://www.soctech.ro/English/Produse/1acizinaft.htm</a> (2) AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: <a href="http://www.amtrade.co.uk/prodinfo.htm">http://www.amtrade.co.uk/prodinfo.htm</a> (3) Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.		
<b>Reliability:</b>	(4) Not assignable. Original source data were not available for review.		
<b>Test Substance:</b>	Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)		
<b>Method/Guideline:</b>	Calculated values using MPBPWIN Version 1.40, a subroutine of the computer program EPIWIN Version 3.10		
<b>Year (guideline):</b>	2000		
<b>Type (test type):</b>	Not applicable		
<b>GLP:</b>	Not applicable		
<b>Year (study performed):</b>	Not applicable		
<b>Test Conditions:</b>	Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10		

Results:	Naphthenic Acid Type	Carbon Number	Molecular Weight	Melting Point, °C
	1-ring cyclopentane	16	254	117
	1-ring cyclohexane	21	325	155
	2-ring cyclopentane	17	266	127
	2-ring cyclohexane	21	323	157
	3-ring cyclohexane	17	264	128
	3-ring cyclohexane	21	321	160
	4-ring cyclohexane	17	262	131
	4-ring cyclohexane	21	319	156

**Remark:** Substances in this category do not have a specific melting point but a range of melting points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual melting point ranges will vary dependent upon their constituent composition.

Melting point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated melting points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products, as evidenced by comparing the estimated melting point values to those cited in product literature and MSDS data (SocTech, S.A. 2003; AGS Chemicals Limited. 2003; Mallinckrodt Baker, Inc. 1997).

**Source:** U.S. EPA. 2000. API (Estimation programs interface) suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

**Reliability:** (2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of melting point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

## 2.2 BOILING POINT

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Not stated

**Year:** Not stated

**Type:** Not stated

**GLP:** Not stated

**Year (study performed):** Not stated

**Test Conditions:** Not stated

<b>Results:</b>	250 °C to 350 °C	Ref (1)
	140 °C to 200 °C	Ref (2)
	200 °C to 370 °C	Ref (3)

**Remark:** Values reported vary widely due to varied composition of the hydrocarbon mixture in naphthenic acids. Values given represent various commercial preparations of naphthenic acids.

**Source:**

- (1) SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: <http://www.socotech.ro/English/Produse/1acizinaft.htm>
- (2) AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: <http://www.amtrade.co.uk/prodinfo.htm>
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**Reliability:** (4) Not assignable

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN<sup>®</sup>, MPBPWIN V1.40 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, melting points were calculated by MPBPWIN, V1.40, EPIWIN V3.10

**Results:** Boiling point values for various cycloaliphatic carboxylic acids in naphthenic acid mixtures are:

Compound	Estimated Boiling Point, °C
C7 cyclohexane	233
C9 dicyclopentane	266
C10 cyclopentane	284
C11 cyclohexane	301
C13 dicyclopentane	326
C14 cyclopentane	340
C15 cyclohexane	352
C17 dicyclopentane	373
C17 tricyclohexane	375

**Remark:** Substances in this category do not have a specific boiling point but a range of boiling points that reflect the hydrocarbon make-up in the naphthenic acid mixtures. Actual boiling point ranges will vary dependent upon their constituent composition.

Boiling point estimates for representative constituents of the naphthenic acid subcategory are listed above. Because naphthenic acids are mixtures of many different isomers of cycloalkyl carboxylic acids, physicochemical properties vary according to the proportions of the individual compounds in their composition. Chemical characterizations of naphthenic acids made by Rogers et al. (2002) demonstrated that these substances have a high degree of compositional heterogeneity, both within and among compounds having different molecular weights and numbers of naphthenic rings.

Estimated boiling points given above represent one to four ring cycloalkyl naphthenic acid structures having molecular weights ranging from approximately 260 to 320. These have been shown to dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands, a source considered to be rich in naphthenic acids (Rogers et al. 2002). In contrast, structural profiles of some commercial naphthenic acid products have been shown to differ substantially from natural extracts (Rogers et al. 2002). Consequently, melting point values given for naphthenic acid extracts from crude oils would be expected to differ from values derived on refined commercial products.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10, subroutine KOWWIN, V 1.66. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

**Reliability:** (2) Reliable with restrictions. Values were estimated using a validated computer model. Estimated values of boiling point for specific molecular structures may not reflect complex mixtures of many different isomeric structures and molecular weights.

## 2.4 VAPOR PRESSURE

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN<sup>®</sup>, MPBPWIN V1.40 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, vapor pressures were calculated by MPBPWIN, V1.40, EPIWIN V3.10

**Results:** Estimated vapor pressures for various naphthenic acid compounds:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Vapor Pressure, Pa
1-ring cyclopentane	16	254	$1.8 \times 10^{-3}$
1-ring cyclohexane	21	325	$1.5 \times 10^{-5}$
2-ring cyclopentane	17	266	$4.8 \times 10^{-4}$
2-ring cyclohexane	21	323	$1.5 \times 10^{-5}$
3-ring cyclohexane	17	264	$4.2 \times 10^{-4}$
3-ring cyclohexane	21	321	$1.4 \times 10^{-5}$
4-ring cyclohexane	17	262	$1.6 \times 10^{-5}$
4-ring cyclohexane	21	319	$4.4 \times 10^{-4}$

**Remark:** A search for pressure values of naphthenic acids failed to uncover reliable information. Product literature data provided narrative phrases such as “very low” or “not applicable” when describing the vapor pressure characteristic for commercial products (SocTech, S.A., 2003; AGS Chemicals Limited. 2003). To gain an understanding of vapor pressure characteristics of naphthenic acids, various hydrocarbon acidic structures reported by Rogers et al. (2002) to predominate in naphthenic acids were estimated for vapor pressure using the EPIWIN<sup>®</sup> computer model (U.S. EPA 2000).

The vapor pressure of complex mixtures is equal to the sum of the vapor pressures of the individual constituents in their pure form times their mole fraction in the mixture (Raoult's Law). Therefore, the total vapor pressure of a complex mixture of naphthenic acids will depend on the proportion of different molecular weight constituents making up the mixture. It is estimated from vapor pressure modeling that representative individual naphthenic acid molecules will have vapor pressure values near or below the measurable limits cited in standard reference guidelines (OECD Guideline 104, Vapor Pressure; OECD, 1995). Hence, based on Raoult's Law, the total vapor pressure of naphthenic acids is expected to be exceedingly low.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

OECD (Organization for Economic Cooperation and Development). 1995. OECD Guideline 104, Vapor Pressure. OECD, Paris, France.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: <http://www.soctech.ro/English/Produse/1acizinaft.htm>

AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: <http://www.amtrade.co.uk/prodinfo.htm>

**Reliability:** (2) Reliable with restrictions  
Estimated vapor pressures were obtained from a validated computer program.

## 2.5 PARTITION COEFFICIENT

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculation, EPIWIN®, KOWWIN V1.66 (U.S. EPA 2000)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, vapor pressures were calculated by KOWWIN, V1.66, EPIWIN V3.10

**Results:** Tabulated values for various naphthenic acid molecules are:

Naphthenic Acid Type	Carbon Number	Molecular Weight	Log Kow
1-ring cyclopentane	16	254	6.7
1-ring cyclohexane	21	325	9.2
2-ring cyclopentane	17	266	6.3
2-ring cyclohexane	21	323	8.3
3-ring cyclohexane	17	264	5.4
3-ring cyclohexane	21	321	7.3
4-ring cyclohexane	17	262	6.5
4-ring cyclohexane	21	319	5.1

<b>Remark:</b>	No partition coefficient measurements were found for naphthenic acids. Therefore, partition coefficients for a range of molecular weight naphthenic acids were estimated using the EPIWIN <sup>®</sup> computer model (U.S. EPA 2000). The partition coefficients reported here span the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected, however, that the lowest molecular weight structures will have the lowest partition coefficients of the compounds in the complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures of molecular weights below 250 will likely show log Kow values lower than those estimated here.
<b>Source:</b>	U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.  Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.
<b>Reliability:</b>	(2) Reliable with restrictions Estimated vapor pressures were obtained from a validated computer program.

## 2.6.1 SOLUBILITY IN DIFFERENT MEDIA

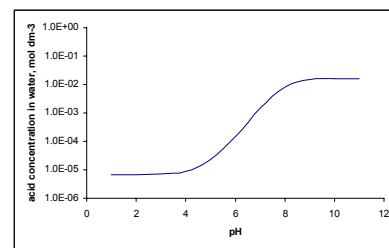
<b>Solubility in:</b>	Water
<b>Test Substance:</b>	Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)
<b>Method:</b>	Calculation, EPIWIN <sup>®</sup> , WSKOWWIN V1.40 (U.S. EPA 2000)
<b>Year:</b>	2000
<b>Type:</b>	Estimation, computer model
<b>GLP:</b>	Not applicable
<b>Year (study performed):</b>	Not applicable
<b>Test Conditions:</b>	Not applicable, water solubility values were calculated by WSKOWWIN, V1.40, EPIWIN V3.10
<b>Results:</b>	Tabulated estimates at 25°C for various naphthenic acid molecular structures are:

<b>Naphthenic Acid Type</b>	<b>Carbon Number</b>	<b>Molecular Weight</b>	<b>Water Solubility, mg/l</b>
1-ring cyclopentane	16	254	0.11
1-ring cyclohexane	21	325	0.0003
2-ring cyclopentane	17	266	0.19
2-ring cyclohexane	21	323	0.002

3-ring cyclohexane	17	264	1.2
3-ring cyclohexane	21	321	0.01
4-ring cyclohexane	17	262	0.08
4-ring cyclohexane	21	319	2.1

**Remark:**

No water solubility measurements were found for naphthenic acids, but their dissociation equilibrium in aqueous systems provides a general understanding of their behavior. These compounds exist as weak acids, with most pKa values being reported at about 5 (Havre, 2002). At low pHs, these compounds exist in their undissociated form and tend to partition onto solids (Rogers et al., 2002). At high pHs, they exist in their dissociated form and become more mobile (Havre, 2002). The following plot shows a theoretical model of the concentration of the acid in the water phase with water pH. This relationship is used as the basis for extraction of naphthenic acids from crude oil, where an alkaline hot water extraction process is used (CEATAG 1998; Brient et al., 1995). However, solubility does not follow an exact acid/base equilibrium, and the equilibrium between oil and water becomes increasingly complex as pH rises. This is due to the tendency of these substances to form micelles and reversed micelles at alkaline pHs. In this system, the existence of 4 or 5 isotropic phases can be observed, making exact solubility measurements difficult (Havre, 2002).



from Havre, 2002

To gain an overview of the water solubility of a range of molecular weight naphthenic acids, the EPIWIN<sup>®</sup> computer model (U.S. EPA 2000) was used to generate solubility estimates for different molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002). It may be expected that the lowest molecular weight structures will have the greatest water solubility of the compounds in complex mixtures. Mixtures of naphthenic acids with a significant proportion of isomeric structures having molecular weights below 250 will likely show water solubilities greater than those estimated here.

**Source:**

U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

Havre, T.E. 2002. Formation of calcium naphthenate in water/oil systems, naphthenic acid chemistry and emulsion stability. Ph.D. Thesis, Department of Chemical Engineering, Norwegian University of Science and Technology, Trondheim, Norway. October 2002.

Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere. 48:519-527.

CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

Brient, J.A., P.J. Wessner, and M.N. Doyle. 1995. Naphthenic acids. In: Kroschwitz, J.I. (ed.). Encyclopedia of Chemical Technology, Vol. 16, 4<sup>th</sup> ed. John Wiley & Sons, Inc., New York. pp 1017 – 1029.

**Reliability:** (2) Reliable with restrictions  
Estimated water solubility values were obtained from a validated computer program.

## 2.14 ADDITIONAL REMARKS

**Memo:** Water solubility of naphthenic acids

**Remark:** Values of water solubility reported in product literature data have varied widely. CEATAG (1998) reported water solubility values of one commercial product to range from 70 mg/l at pH 0.91 to 5040 mg/l at pH 9.16. Other product data sources for water solubility report narrative phrases such as “very low water solubility” (SocTech S.A., 2003), “not applicable” (Mallinckrodt Baker Inc., 1997), or “only slightly soluble in water” (AGS Chemicals Limited, 2003).

**Source:** CEATAG (Conrad Environmental Aquatic Technical Advisory Group). 1998. Naphthenic acids background information discussion report. Alberta Department of Energy, Edmonton, AB.

SocTech, S.A. 2003. Product Data Sheet, Naphthenic Acids. Web Version URL: <http://www.soctech.ro/English/Produse/1acizinaft.htm>

AGS Chemicals Limited. 2003. Material Safety Data Sheet, Naphthenic Acid. Web Version URL: <http://www.amtrade.co.uk/prodinfo.htm>

Mallinckrodt Baker, Inc. 1997. Material Safety Data Sheet No. N0310, Naphthenic Acids (CAS No. 1338-24-5). Mallinckrodt Baker Inc., Phillipsburg, New Jersey.

**Reliability:** (4) Not assignable. Data were obtained from secondary literature sources.

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### 3. Environmental Fate Data

#### 3.1.1 PHOTODEGRADATION

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Calculations by EPIWIN<sup>®</sup> V3.10; Subroutine AOPWIN V1.90.

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** Not applicable, photodegradation potential was calculated by AOPWIN, V1.90, EPIWIN V3.10

**Results:**

Type	Carbon Number	Molecular Weight	Half Life (days)
1-ring cyclopentane	16	254	0.6
1-ring cyclohexane	21	325	0.4
2-ring cyclopentane	17	266	0.5
2-ring cyclohexane	21	323	0.3
3-ring cyclohexane	17	264	0.3
3-ring cyclohexane	21	321	0.3
4-ring cyclohexane	17	262	0.3
4-ring cyclohexane	21	319	0.3

**Remark:** AOPWIN V1.90 calculates atmospheric oxidation rate constants between photochemically produced hydroxyl radicals and organic chemicals. These rate constants are then used to calculate half lives for those compounds based on average atmospheric concentrations of hydroxyl radicals and ozone. Atmospheric oxidation rates were calculated for a range of molecular structures covering a range of molecular weights and ring structures that were reported to predominate in Athabasca oil sands extracts (Rogers et al., 2002).

Although the low vapor pressures of these base oils indicate that volatilization will not be a very significant fate process, oxidation half-lives indicate that any vapors emitted to the troposphere would be rapidly oxidized and not persist in the atmosphere.

**Source:** U.S. EPA. 2000. EPI (Estimation Programs Interface) Suite, V 3.10. US Environmental Protection Agency, Office of pollution prevention and toxics, Washington DC.

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Rogers, V.V., K. Liber, and M.D. MacKinnon. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. *Chemosphere*. 48:519-527.

**Reliability:** (2) Reliable with restrictions  
Estimated water solubility values were obtained from a validated computer program.

### 3.1.2 STABILITY IN WATER

**Remark:** Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). The chemical components found in the materials that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

**Source:** Harris, J.C. 1982. Rate of hydrolysis. In; Handbook of Chemical Property Estimation Methods. W.L. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds. McGraw-Hill Book Co., New York, NY.

### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

**Test Substance:** Naphthenic Acids (CAS Nos. 001338-24-5; 061790-13-4; 064754-89-8)

**Method:** Level 1 Fugacity-Based Environmental Equilibrium Partitioning Model (Version 2.11)

**Year:** 2000

**Type:** Estimation, computer model

**GLP:** Not applicable

**Year (study performed):** Not applicable

**Test Conditions:** The EQC Level I is a steady state, equilibrium model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment.

**Results:** Air / Water / Soil / Sediment / Suspended Sediment / Biota

**Type (C-number)(Molecular Weight)**

**Distribution In:**

	Air	Water	Soil	Sediment	Suspended Sediment	Biota
1-ring cyclopentane (C16)(254)						
	<0.1	<0.1	98	2	<0.1	<0.1
1-ring cyclohexane (C21)(325)						
	<0.1	<0.1	98	2	<0.1	<0.1
2-ring cyclopentane (C17)(266)						
	<0.1	<0.1	98	2	<0.1	<0.1
2-ring cyclohexane (C21)(323)						
	<0.1	<0.1	98	2	<0.1	<0.1
3-ring cyclohexane (C17)(264)						
	<0.1	0.4	97	2	<0.1	<0.1
3-ring cyclohexane (C21)(321)						
	<0.1	<0.1	98	2	<0.1	<0.1
4-ring cyclohexane (C17)(262)						
	<0.1	<0.1	98	2	<0.1	<0.1
4-ring cyclohexane (C21)(319)						

**Remark:** Multimedia distribution was calculated for a range of naphthenic acids covering predominant molecular weight and ring structures of such constituents found in Athabasca oil sands extracts (Rogers et al., 2002). The principle distribution of these constituents following an environmental release would be to soil and/or sediment, with overwhelming partitioning to soil.

**Source:** Mackay, D. 1991. Multimedia environmental models; The fugacity approach Lewis Publ. CRC Press, Boca Raton, Florida.

**Reliability:** (2) Reliable with restrictions  
Estimated environmental distribution was obtained from a validated computer program.

### 3.5 BIODEGRADATION

**Remark:** No standardized testing for ready or inherent biodegradation was found for naphthenic acids. Results of relevant scientific journal articles on the biodegradability of naphthenic acids are reviewed in Section 3.8

### 3.8 ADDITIONAL REMARKS

**Memo:** Biodegradation of naphthenic acids

**Remark:** Herman et al. (1993) conducted four experiments on the biodegradation of specific cycloalkane carboxylic acids:

**Experiment No. 1.** Biodegradation of four naphthenic acid compounds (cyclopentane carboxylic acid, CCP; cyclohexane carboxylic acid, CCH; 1-methyl-1-cyclohexane carboxylic acid, 1MCCH; and 2-methyl-1-

cyclohexane carboxylic acid, 2MCCH) was measured in pore water from Athabasca oil sands tailings ponds. The purpose of the tailings ponds was to serve as a settling basin to separate solids from liquid generated during the extraction of acidic compounds from bitumen. Therefore, the tailings ponds were considered to harbor indigenous microorganisms adapted to naphthenic acids. The collected pore water was centrifuged and filtered and served as the nutrient medium. Inoculum was 0.5 ml of the original oil sands tailings sample. Duplicate flasks containing 30 ml of medium were spiked with 1-ml aliquots of stock solutions of the different naphthenic acids to achieve a final concentration of 1000 mg/l. Test flasks received the inoculum and control flasks received inoculum in which the microbes had been heat-killed. One set of duplicate flasks received a nutrient addition in the form of  $\text{NH}_4\text{NO}_3$ ,  $\text{K}_2\text{HPO}_4$ , and  $\text{KH}_2\text{PO}_4$  to a final concentration of 0.2 g/l of each compound. The flasks were incubated at room temperature on a rotary shaker. After 0, 3, 6, 9, 16, 26, and 40 days, a 3-ml sample was removed, centrifuged, and filtered through a 0.2 micron syringe filter. The samples were analyzed for the test compounds by gas chromatography equipped with a flame ionization detector. Peak areas were converted to concentration using a calibration curve for each compound.

**Results of Experiment 1.** The bacterial populations of oil sands tailings was shown to have the metabolic capability of degrading carboxylated cycloalkanes as shown in the following table of results.

Day	CCP		CCH		1MCCH		2MCCH	
	NP-	NP+	NP-	NP+	NP-	NP+	NP-	NP+
0	100	42	100	68	100	100	100	100
6	100	5	100	12	100	100	100	100
10	100	0	100	1	100	100	100	100
16	100	0	100	0	100	100	100	100
26	100	0	100	0	100	100	100	49
40	100	0	100	0	100	100	100	0

Using tailings pond water as a growth medium, degradation of CCP, CCH, and 2MCCH was achieved only if nutrients were added to the medium. CCP and CCH were degraded rapidly, within one week, while methylated carboxylic acids were more resistant to biodegradation. 2MCCH was degraded within 40 days, but no degradation was observed for 1MCCH.

**Experiment No. 2.** Triplicate tailings pond microcosms were created using 200 ml of the tailings sample (as inoculum and medium) in 500-ml Erlenmeyer flasks closed with cotton stoppers. A filter-sterilized solution of CCP and 1MCCH was added to each microcosm for a final concentration of 1000 mg/l. Sterile controls were autoclaved and also spiked with the test compounds. Microcosms were incubated at room temperature on a rotary shaker. After 1, 2, 3, 4, 6, and 9 weeks, samples were removed and analyzed for CCP and 1MCCH by GC.

**Results of Experiment No. 2.** Biodegradation of CCP was complete within the first week. No biodegradation of 1MCCH was evident after six weeks. At the six-week period, nitrogen and phosphorus was added

whereby complete biodegradation of 1MCCH was noted following between the 6 and 9-week sampling. No 1MCCH was measured at 9 weeks. Neither CCP nor 1MCCH was degraded in the control microcosms.

**Experiment No. 3:** Tailings pond bacteria were isolated on agar plates and colony types were examined for their ability to utilize carboxylated cycloalkanes as their sole carbon source. Individual colonies were inoculated into a solution of carboxylated cycloalkanes (1000 mg/l) in modified Bushnell and Haas (MGH) minimal salts medium. The ability of the isolate to metabolize the carbon source was monitored by GC analysis. In a second part to this experiment, a carboxylate-degrading mixed bacterial culture was enriched from the tailings pond sample using standard procedures. The mixed culture was maintained on a mixture of CCP, 1MCCH, and 2MCCH (500 mg/l each) in MBH with yeast extract (1000 mg/l) added as a supplemental carbon source.

**Results of Experiment No. 3.** Of 10 separate colony types isolated from oil sands tailings, one colony type was found to utilize CCP and CCH as its sole carbon source. The isolate was a Gram negative, non-motile, catalase positive, oxidase negative, non-fermenting, aerobic rod, and was identified as an *Acinetobacter* sp. The isolate rapidly degraded CCP and CCH, with complete loss of substrate from the medium within 2 weeks of incubation. However, this isolate was unable to degrade methyl-substituted cyclohexane carboxylic acids. The mixed bacterial culture enriched from the tailings pond sample on a mixture of carboxylated cycloalkanes was found to degrade 1MCCH and 2MCCH, but only when the medium was supplemented with yeast extract. After a 2-week incubation period, the mixed culture had degraded 100% of the 1MCCH and 67% of the 2MCCH.

**Experiment No. 4.** Radiolabeled hexadecane was spiked into the maltene fraction of pure bitumen. Hexadecane mineralization experiments were performed using 5 ml of oil sands tailings in 60-ml serum vials and inoculated with 10  $\mu$ l of spiked maltene. One set of vials received nutrient addition as described before. Sterile controls were autoclaved before the addition of the labeled hydrocarbon. Mineralization was determined from triplicate vials after 5, 10, 16, 27, and 40 days using the closed-loop trapping system. Radioactivity was measured using a scintillation cocktail and a Beckman LS8000 scintillation counter.

**Results of Experiment No. 4.** The results of hexadecane mineralization within oil sands tailings showed that the biodegradation of an n-alkane was nutrient limited. Percent biodegradation reached 50% by day 16 and maintained a plateau through day 40.

**Conclusions.** This study showed the potential for biodegradation of naphthenic acids by investigating the biodegradation of both carboxylated cycloalkanes and hexadecane. Although natural naphthenic acids present in oil sands tailings have greater structural complexity than the compounds examined in this study, the results show the potential for both for biodegradation of the alkyl side chain and the carboxylated cycloalkane ring components of naphthenic acids. Biodegradation potential was reduced by methyl substitution on the

cycloalkane ring, although these compounds could be degraded with the addition of mineral nutrients.

**Source:** Herman, D.C., P.M. Fedorak, and J.W. Costerton. 1993. Biodegradation of cycloalkane carboxylic acids in oil sand tailings. *Can. J. Microbiol.* 39:576-580.

**Reliability:** (2) Reliable with restrictions. The report was a well-documented study that meets basic scientific principles.

**Memo:** Biodegradation of cycloalkane carboxylic acids in oil sand tailings

**Remark:** Herman et al. (1994) investigated the ability of microbial populations indigenous to oil sands tailings to biodegrade solutions of natural naphthenic acids from oil sands tailings and commercial naphthenic acid sodium salts (Kodak Chemicals).

Four experiments were run:

- 1) Evaluation of mineralization of naphthenic acids sodium salts (NAS) and oil sands tailings extracts of naphthenic acids (TEX),
- 2) Evaluation of mineralization of four model naphthenic acid compounds, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA), 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and *trans*-4-pentylcyclohexane carboxylic acid (4PCCA),
- 3) Gas chromatographic analysis of NAS and TEX biodegradation, and
- 4) Respirometry measurements of cyclohexane pentanoic acid, NAS, and TEX in tailings microcosms.

**Test Substances:** Test substances used in the four experiments included the following materials: 1) Tailings water extract (TEX), 2) commercial sodium naphthenate mixture (NAS), and 3) pure compound naphthenic acids, cyclohexane carboxylic acid (CCA), cyclohexane pentanoic acid (CPA), 2-methyl-1-cyclohexane carboxylic acid (2MCCA), and *trans*-4-pentylcyclohexane carboxylic acid (4PCCA).

**Inoculum:** Inoculum used in the biodegradation experiments was NAS- and TEX- degrading enrichment cultures derived from oil sands tailings water. These cultures were created by diluting a 10-ml sample of oil sands tailing into 90 ml of mineral salts medium that contained either NAS (100 mg/l) or TEX (1:50 dilution). The mineral salts medium was modified Bushnell-Haas medium. Successive transfers 1% v/v of the enrichment culture into fresh NAS- to TEX-containing medium were on monthly basis and incubated at room temperature on a gyratory shaker (100 rpm). The viable cell number within each enrichment culture was estimated using the plate count technique.

**Experiment No. 1.** A measurement of CO<sub>2</sub> production was used to evaluate the ability of the enrichment cultures to mineralize components within both the NAS and TEX mixtures. Mineralization experiments were performed using 60-ml serum bottles containing 15 ml of growth medium. The growth medium consisted of sterilized mineral salts medium with NAS (100 mg/l) or TEX (1:20 and 1:50 dilutions) as the sole carbon source. Dissolved organic carbon analyses showed that 100 mg/l of NAS contained 60 mg C/l, while 1:20 and 1:50 dilutions of TEX contained 50 and 21 mg C/l, respectively. The serum bottles were

inoculated with 0.15 ml of either the NAS-degrading or the TEX-degrading enrichment culture, sealed with rubber stoppers, and incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 6-day intervals over 24 to 30 days, three inoculated bottles and one control (inoculated but lacking NAS or TEX) were acidified to pH <2 using 1 ml of 1M H<sub>2</sub>SO<sub>4</sub> to convert all forms of inorganic carbon into CO<sub>2</sub>. A 0.5 ml headspace sample from each bottle was analyzed for CO<sub>2</sub> content by gas chromatography. Mineralization of the organic substrate was first corrected for the amount of CO<sub>2</sub> in the control bottles, then expressed either as the total amount of CO<sub>2</sub> produced within the bottle or as the percentage of organic carbon converted to CO<sub>2</sub>.

**Results of Experiment No. 1.** The mineralization studies showed that the NAS- and TEX-degrading enrichment culture was capable of mineralizing components within both the NAS and TEX mixtures. The percentage of organic carbon converted to CO<sub>2</sub> by the NAS-degrading culture was 48% (day 24) in the NAS bottles and 20% (day 20) in the TEX bottles. The percentage of organic carbon converted to CO<sub>2</sub> by the TEX-degrading culture was 34% (day 30) for the TEX bottles and 20% (day 25) for the NAS bottles.

**Experiment No. 2.** Mineralization of the four model naphthenic acid compounds was measured as the amount of CO<sub>2</sub> evolved from incubating solutions of the compounds dissolved in nutrient medium and inoculated with enrichment cultures of NAS-degrading microorganisms, TEX-degraders, or oil sands tailings pond water (TPW). Fifteen milliliters of 1 mM solutions of the compounds dissolved in mineral salts medium were placed in 60-ml serum bottles and inoculated (1% v/v) with the different sources of microbes then sealed with rubber stoppers. Bottles were incubated at room temperature on a gyratory shaker (100 rpm). After 3, 6, 12, and 24 days, duplicate bottles were acidified and headspace CO<sub>2</sub> determined by GC. The level of CO<sub>2</sub> production was corrected for the amount of CO<sub>2</sub> within the control bottles and expressed as the percentage of organic substrate converted to CO<sub>2</sub>.

**Results of Experiment No. 2.** The following results were obtained:

Mineralization by day 24, % organic C converted to CO<sub>2</sub>:

<b>Substrate</b>	<b>NAS-degraders</b>	<b>TEX-degraders</b>	<b>TPW</b>
CCA	41	56	57
CPA	45	57	58
2MCCA	47	7	67
4PCCA	6	24	24

**Experiment No. 3.** A 1% (v/v) inoculum of the NAS-degrading enrichment culture was placed in 125-ml Erlenmeyer flasks containing 50 ml of either NAS (30 mg/l) or TEX (1:50 dilution) in mineral salts medium. Control flasks received inoculum of heat-killed cells. The flasks were incubated at room temperature on a gyratory shaker (100 rpm). After an incubation period of 4, 8, and 16 days for NAS and 6, 12, and 24 days for TEX, the contents of two flasks and two control flasks were extracted for GC analysis. Samples were extracted and the carboxylic acids were derivatized to methyl esters prior to analysis.

Derivatized extracts were analyzed by GC with a capillary column and flame ionization detector.

**Results of Experiment No. 3.** Chromatographic analysis of solution from the control flasks revealed an unresolved series of many overlapping peaks that created a hump in the GC profile. When the mixture that was inoculated with NAS-enrichment culture, a reduction in the size of the hump was evident within 4 days, indicating that components within the naphthenic acid mixture were being degraded. Chromatographic analysis of the TEX samples revealed a similar hump of many overlapping peaks that appeared in the NAS GC profile. Biodegradation of TEX by the NAS-degrading culture did not result in a noticeable reduction in the size of the hump associated with TEX, despite evidence of mineralization of components within the mixture.

**Experiment No. 4.** A measurement of CO<sub>2</sub> production and O<sub>2</sub> utilization within sealed microcosms was used to monitor microbial activity in samples of TPW, and to determine the effect of nutrient addition (N and P) or carbon substrate addition (cyclohexane pentanoic acid (CPA), sodium salts of naphthenic acids (NAS), or tailings pond extracts of carboxylic acids (TEX)) on the level of microbial activity within TPW.

60 ml of TPW was placed into sterile 125-ml Erlenmeyer flasks, sealed with rubber stoppers in which a sampling port had been drilled and then sealed with clear silicone. Nutrients in the form of N and P were added. Carbon substrates (CPA, NAS or TEX) were added as a filter-sterilized solution to create a final concentration of 60 mg organic carbon/l. All flasks were incubated at room temperature on a gyratory shaker (100 rpm). At 3 to 80 day intervals, 0.5 ml of headspace was sampled and analyzed for CO<sub>2</sub> and O<sub>2</sub> using GC. Following 5 weeks of incubation, the contents of the flasks containing CPA were extracted and analyzed using the procedure described for the GC analysis in experiment 3.

**Results of Experiment No. 4.** The addition of CPA to TPW resulted in increased microbial activity, as indicated by greater levels of CO<sub>2</sub> production and O<sub>2</sub> utilization when compared with TPW alone. Sterilized TPW demonstrated no CO<sub>2</sub> production or O<sub>2</sub> utilization. Even greater levels of microbial activity were evident when N and P were added in addition to CPA, indicating that mineralization could be enhanced by the addition of mineral nutrients. GC analysis of CPA in TPW microcosms after 35 d of incubation revealed that the concentration of CPA was below the level of detection in 2/3 microcosms and reduced 10-fold in the third microcosm. There was no detectable CPA in the three N and P-amended microcosms.

Similarly, NAS and TEX additions to microcosms increased microbial activity in TPW, although microbial activity was enhanced by the addition of N and P. Increases in both CO<sub>2</sub> evolution and O<sub>2</sub> utilization were seen.

**Conclusions.** This investigation showed that naphthenic acids, either as a commercial preparation of sodium salt (NAS) or natural extracts from oil sands tailing water (TEX) are capable of being utilized by natural assemblages of microorganisms. Addition of nitrogen and

phosphorus enhances the utilization of these substrates by the microbes.

**Source:** Herman, D.C., P.M. Fedorak, M.D. MacKinnon, and J.W. Costerton. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oils sands tailings.

**Reliability:** (2) Reliable with restrictions. The report was a well-documented study that meets basic scientific principles.

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## 4. Ecotoxicity

### 4.1 ACUTE/PROLONGED TOXICITY TO FISH

Test Substance:	Naphthenic acids
Method/Guideline:	Hart, et al. 1945; Doudoroff et al. 1951
Year (guideline):	N/A
Type (test type):	Static
GLP:	No
Year (study performed):	1965
Species:	zebra fish ( <i>Brachydanio rerio</i> )
Analytical Monitoring:	No
Exposure Period:	96 hours
Statistical Method: (FT - ME)	Graphical interpolation for determining the LC50.
Test Conditions: (FT - TC)	Test containers were 2.5 gallon aquariums, each fitted with an air stone through which compressed air was bubbled to maintain a 5-9 ppm dissolved oxygen concentration in the dilution water. The aquariums were maintained at a temperature of 24 +/- 1 °C. Dilution water was synthetic soft water prepared with distilled water and ACS grade chemicals.
<ul style="list-style-type: none"><li>Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.</li></ul>	<p>The lot of test fish displayed no visible disease. The average size was 3.2 cm total length. Before testing the fish were acclimated to the dilution water for 5 days. During the acclimation period they were fed <i>Daphnia</i> and white worms, but were not fed for 36 hours before or during the testing.</p> <p>Test concentrations were prepared by direct addition of the test substance to the test chambers followed by mixing. Test concentrations were control, 7.5, 8.7, 10, 11.5, 13.5, 15.5, 18.0, 21.0, and 24.0 ppm naphthenic acids. After the test solutions were prepared, ten fish were placed in each test container. Controls were run in duplicate, while test levels were run singly. Mortality was evaluated at 24, 48, and 96 hours, and the criteria for death was a cessation of gill movement and failure to respond to mechanical stimulus.</p> <p>Following the 96 hour test period the TLm (median tolerance limit) was determined from visual observation of the dose-response pattern. Where no exact TLm response resulted, the TLm was interpolated from a plot of the concentration and survival data on semi-log paper.</p>
Results: (FT - RS)	96-hour TLm = 16.3 ppm
Units/Value:	

The following dose-response data were provided:

Concentration of Naphthenic acids, ppm	Number Tested	% Dead at 96 hours
0 (control #1)	10	0
0 (control #2)	10	0
7.5	10	0
8.7	10	40

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10	10	20
11.5	10	0
13.5	10	20
15.5	10	30
18	10	80
21	10	100
24	10	100

- **Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**

The article reported that pH and dissolved oxygen concentrations were taken during the test, but these data were not reported.

**Conclusion: (FT - CL)**

**Reliability: (FT - RL)**

(2) Reliable with restrictions. The test was conducted under referenced test conditions current for the period in which the study was run. The report provided sufficient details for assessment.

**Source: (FT - RE)**

Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios *Brachydanio rerio* (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish *Lepomis macrochirus* Raf. *Notulae Naturae*. No. 381:1-9.

Hart, W.B., P. Doudoroff, and J. Greenbank. 1945. The evaluation of the toxicity of the industrial wastes, chemicals and other substances to freshwater fishes – The Atlantic Refining Company, Philadelphia, PA. 315 pp.

Doudoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galstoff, W.B. Hart, T. Patrick, E.R. Strong, E.W. Surber, and W.M. VanHorn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. *Sew. and Ind. Wastes*. 23(11):1380-1397.

**Other (source): (FT - SO)**

FT - Freetext  
ME - Method  
TC - Test Conditions  
RS - Results  
CL - Conclusion  
RL - Reliability  
RE - Reference  
SO - Source

**Test Substance:** Naphthenic acid mixture (commercially available from Eastman Chemicals)  
**Method/Guideline:** Peltier and Weber 1985  
**Year (guideline):** 1985  
**Type (test type):** static acute  
**GLP:** not stated  
**Year (study performed):**  
**Species:** three-spine stickleback (*Gasterosteus aculeatus*)  
**Analytical Monitoring:** no

**Exposure Period:** 96 hours

**Statistical Method:** (FT - ME)

**Test Conditions:** (FT - TC)

- Note: Concentration prep., vessel type, volume, replication, water quality parameters, environmental conditions, organisms supplier, age, size, loading.**

**Summary of Test Conditions**

Organism age: juvenile  
Test Temperature: 20 °C +/- 2 °C  
Photoperiod: 16 h light/8 h dark  
Light intensity: 10 – 50 micro-einsteins  
Light quality: wide spectrum fluorescent  
Test container: 5 gallon aquaria  
Dilution water: Carquinex Strait  
Test Volume: 15 liters  
Animals per container: 10  
Replicate containers: 2  
Number of concentrations: 6 (5 concentrations and a control)  
Food: none  
Test duration: 96 h  
Test endpoint: mortality  
Salinity: 15 parts per thousand  
Test pH: ambient  
Test article: Martinez Refinery effluent (non-toxic) with added naphthenic acids

Test solutions were prepared by creating a 1 percent solution using non-toxic effluent pH adjusted to 12 with sodium hydroxide. The stock solution was mixed overnight prior to use. The stock solution was used to spike non-toxic treated effluent to nominal naphthenic acid concentrations from 2.5 to 30 mg/l.

Test organisms were held at least seven days prior to testing in dilution water. During testing at 24-h intervals, the salinity, temperature, pH, and dissolved oxygen were measured in all control and test tanks. Survival data were taken at 24-h intervals and dead individuals were removed when observed.

**Results:** (FT - RS)

**Units/Value:**

LC50 estimated to be in the range of 5 mg/l.

The following dose response data were reported:

<u>Concentration (mg/l)</u>	<u>% Survival</u>
0 (control)	100
2.5	60
5	10
10	0
15	0
30	0

- Note: Deviations from protocol or guideline, analytical method, biological observations, control survival.**

Although an LC50 could have been calculated using contemporary methods, the author elected to estimate its value. The report stated that water chemistry data were collected but no data were reported.

**Conclusion:** (FT - CL)

**Reliability:** (FT - RL)

(2) Reliable with restrictions. A statistically-defined LC50 was not calculated. Water chemistry data were not reported.

**Source:** (FT - RE)

Dorn, P.B. 1992. Case Histories – The petroleum refining industry. In: Ford, D.L. (ed.). Water Quality Management Library, Volume 3, Toxicity Reduction Evaluation

and Control. Technomic Publishing Co., Lancaster, PA. pp 183 – 223.

Peltier, W.H., and C.I. Weber, eds. 1985. Method for measuring acute toxicity of effluents to freshwater and marine organisms, 3<sup>rd</sup> edition. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-85-014. 230 pp.

Stephan, C.E. 1977. Method for calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA. pp 65-84.

**Other (source): (FT - SO)**

FT - Freetext  
ME - Method  
TC - Test Conditions  
RS - Results  
CL - Conclusion  
RL - Reliability  
RE - Reference  
SO - Source

## 4.9 ADDITIONAL REMARKS

**Memo:** Effect of naphthenic acids on survival of zebra fish (*Brachydanio rerio*) embryos

**Remark:** Zebra fish embryos were exposed for 48 hours to a range of naphthenic acids concentrations to determine the TLm (median tolerance limit) for embryo survival. Embryos were collected from a culture unit once they attained Stage 21 as designated by Hisaoka and Battle (1958). Ten embryos were exposed to each test solution and control in petri dishes holding 45 ml of the exposure solutions. Exposure solutions were prepared by diluting a stock solution of naphthenic acids (100 mg naphthenic acids in 50 ml acetone) with water. In addition to a control group, nine concentrations of naphthenic acids were prepared at 2.4, 3.2, 4.2, 6.5, 10, 15.5, 24, 32, and 42 ppm naphthenic acids. Mortality was assessed at 24 and 48 hours of exposure. The embryo was considered dead if it had an opaque appearance.

A TLm of 3.5 ppm was obtained by plotting the survival versus concentration on semilog paper and interpolating the 50% survival concentration. The following dose response was given:

Test Concentration, ppm	Percent Dead
0 (control)	0
2.4	0
3.2	40
4.2	70
6.5	100
10	100
15.5	100
24	100
32	100
42	100

**Source:** Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios *Brachydanio rerio* (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish *Lepomis macrochirus* Raf. *Notulae Naturae*. No. 381:1-9.

Hisaoka, K.K., and H.I. Battle. 1958. The normal development stages of the zebra-fish, *Brachydanio rerio* (Hamilton-Buchanan). *J. Morph.* 102(2):311-327.

**Reliability:** (2) Reliable with restrictions. Although the test was conducted prior to the time of standardized test methods, the report provided sufficient information on the dose-response pattern for the test substance.

**Memo:** Effect of naphthenic acids on survival of bluegill (*Lepomis macrochirus*)

**Value:** 48-hour TLm = 5.6 mg/l naphthenic acids

**Remark:** The value was reported in a summarized journal article (Cairns et al., 1965) as originating in Cairns and Scheier (1962).

**Source:** Cairns, J. Jr., A. Scheier, and J.J. Loos. 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios *Brachydanio rerio* (Hamilton-Buchanan) and zebra danio eggs with that of adult bluegill sunfish *Lepomis macrochirus* Raf. *Notulae Naturae*. No. 381:1-9. Acad. Nat. Sci. Philadelphia.

Cairns, J. Jr., and A. Scheier. 1962. The effect of temperature and hardness of water upon the toxicity of naphthenic acids to the common bluegill (*Lepomis macrochirus* Raf.) and the pond snail (*Physa heterostrophs* Say). *Notulae Naturae*. No. 353: 111 pp. Acad. Nat. Sci. Philadelphia.

**Reliability:** (3) Not reliable. The endpoint was cited in the text of a journal article without details of the test.

**Memo:** Effect of naphthenic acids on survival of bluegill (*Lepomis macrochirus*)

**Value:** 96-hour LC50 = 0.0026 mg/l

**Remark:** Test chambers were 30x60x30 cm all-glass vessels. Dilution water was well water. Testing was performed at a temperature of 22 +/- 1°C under a 16-h light/8-h dark photoperiod.

The test included five concentrations of the test substance and a dilution water control. Each test level included 20 fish distributed 10 each to two replicate chambers per treatment.

Dissolved oxygen ranged from 4.3 to 8.1 mg/l, pH ranged from 7.4 to 8.0, and temperature ranged from 22 to 24 °C when measured daily during the test. Specific conductance between the test solutions remained constant at 550 (no units given) when measured at the beginning of the test.

The report stated that serial dilutions of the test product were created for testing, although no details were given as to how the serial dilutions or the original solution was created. The raw data indicated that concentrations were expressed as a percent, while the LC50 and confidence interval was reported as parts per million. There was no explanation how the values for percent were related to parts per million.

Critical details of testing procedures and animal culture were omitted from the report.

**Source:** Exxon Corporation. 1980. Aquatic bioassay testing of Exxon Corporation's experimental compounds (MRD 78-100). Report by Battelle Columbus Laboratories, Columbus, Ohio.

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## **5. Acute Toxicity**

### **5.1.1 ACUTE ORAL TOXICITY**

<b>Type:</b>	LD <sub>50</sub>
<b>Value:</b>	5.88 (4.31-8.02) g/kg bw
<b>Species:</b>	Rat
<b>Strain:</b>	Wistar
<b>Sex:</b>	Male
<b>Number of Animals:</b>	5 per dose level (7 dose levels)
<b>Vehicle:</b>	None – administered undiluted
<b>Year:</b>	1979
<b>GLP:</b>	Unable to determine
<b>Test Substance:</b>	MRD-79-10 (Raw naphthenic acid derived from kerosene )
<b>Method</b>	<p>Seven groups of 5 male rats were dosed at 1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg of body weights. Food and water were freely available except for the 16-20 hours prior to dosing.</p> <p>The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.</p>
<b>Result:</b>	<p>Deaths occurred at the four highest dose levels: 3.26, 4.64, 6.81, and 10 g/kg bw. 8/10 animals died at the two highest dose levels. Significant predeath toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died during the study included dilated hearts and gastrointestinal irregularities.</p> <p>The LD<sub>50</sub> was determined to be 5.88 (4.31-8.02) g/kg bw</p>
<b>Reliability:</b>	(1) Reliable without restrictions; appears to be comparable to a guideline study with adequate experimental details provided; although the investigators used male rats only, there is sufficient experimental detail to make a conclusion on the study's validity, and the results can be used to assess the potential acute toxicity of naphthenic acid.

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<b>Source</b>	Exxon, Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, 1979.
<b>Type:</b>	LD <sub>50</sub>
<b>Value:</b>	3.0 g/kg bw (fraction from crude kerosene acids) 5.2 g/kg bw (fraction from mixed crude oils)
<b>Species:</b>	Rat
<b>Strain:</b>	No information
<b>Sex:</b>	No information available
<b>Number of Animals:</b>	"Sufficient animals ...so the the LD50 dose could be computed by either the Weil or the Litchfield and Wilcoxon method"
<b>Vehicle:</b>	None – administered undiluted
<b>Year:</b>	1955
<b>GLP:</b>	Unable to determine
<b>Test Substance:</b>	1) 7-93% Naphthenic acid fraction from crude kerosene acids 2) 65-69% Naphthenic acid fraction from mixed crude oils
<b>Method</b>	"The LD50 ..was determined in rats by use of screening test procedures similar to those of Smyth and Carpenter." (Smyth, H.F., and C.P. Carpenter. 1944. Place of the range finding test in the industrial toxicology laboratory. J. Indust. Hyg. & Tox. 26: 269.
<b>Result:</b>	Death appears to result from gastrointestinal disturbances, with the mortality peak occurring on the third to fourth day after administration. The animals exhibited anorexia, inanition, diarrhea, and asthenia.  The LD <sub>50</sub> s were determined to be 3.0 g/kg bw (fraction from crude kerosene acids) and 5.2 g/kg bw (fraction from mixed crude oils)
<b>Reliability:</b>	(2) Reliable with restrictions; Although not a guideline or GLP study, and some of the experimental details are not available, the study does appear to be well-conducted, and cites that the investigators followed published methodologies for conducting a statistically valid LD50. The data are supportive of other acute toxicity studies reported by Exxon and Pennisi.
<b>Source</b>	Rockhold, W.T. 1955. The toxicity of naphthenic acids and their metal salts. Archs Ind Hlth 12, 477-482.

**Type:** LD<sub>50</sub>  
**Value:** 3550 mg/kg bw  
**Species:** Mice  
**Strain:** White – no other information  
**Sex:** Male  
**Number of Animals:** No information available  
**Vehicle:** No information available  
**Year:** 1977  
**GLP:** Unlikely  
**Test Substance:** Naphthenic Acid – no further description  
**Method** Not described  
**Result:** Oral administration resulted in 1) CNS depression without analgesia and no loss of corneal reflex, 2) corneal eye opacity, 3) dryness of mouth, 4) convulsions, 5) diarrhea, and 6) death due to respiratory arrest.  
**Reliability:** (4) Not assignable. This information is taken from a published, meeting abstract. The level of experimental details provided is not sufficient to verify the conclusions.  
**Source** Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181.

**Type:** Acute Oral Toxicity Study (Not LD50)  
**Value:** Not applicable  
**Species:** Rat  
**Strain:** Wistar  
**Sex:** Male/Females  
**Number of Animals:** 10 Females/dose (3 doses, plus control)  
10 Males/dose (1 dose, plus control)  
**Vehicle:** Aqueous solutions of naphthenic acids/Water  
**Year:** 2002

<b>GLP:</b>	Unable to determine
<b>Test Substance:</b>	Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 55,080, 5508 or 550.0 mg/l naphthenic acids – derived from athabasca sands sands tailings.
<b>Method</b>	<p>Female rats were given a single oral dose of naphthenic acids at 3, 30 or 300 mg/kg bw, while male rats received 300 mg/kg. Control animals were given tap water. All animals were monitored continuously for 12 hr after dosing, and thereafter daily. Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination.</p> <p>Statistical analysis was performed by using a one-way ANOVA to compare means of female dose and control groups with respect to consumption, body weights, and organ weights. A pair wise multiple comparison test was then used in cases where statistical significance was reached. For the male dose and control groups, a Student's t-test was used to compare group means. Probability values of <math>p \leq 0.05</math> was considered statistically significant.</p>
<b>Result:</b>	<p>The following effects were seen in the high dose groups:</p> <ul style="list-style-type: none"><li>• Decreased food consumption immediately following dosing.</li><li>• Lethargy and mild ataxia (2/10 females, 3/10 males)</li><li>• Statistically significant increase relative organ weights: ovaries, spleen in females- testes, heart in males</li><li>• 7/10 females and 6/10 males exhibiting eosinophilic pericholangitis</li><li>• 6/10 males and 2/10 females with brain hemorrhage.</li></ul> <p>The following effects were seen in the mid dose group:</p> <ul style="list-style-type: none"><li>• 7/10 females and 4/10 males with heart lesions.</li></ul>
<b>Reliability:</b>	(2) Reliable with restriction. The study is not an acute toxicity study as defined by OECD SIDS/HPV, however it appears to be well conducted and provides additional information regarding potential acute, non-lethal effects of naphthenic acids following oral exposure.
<b>Source</b>	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.

#### 5.1.2 ACUTE DERMAL TOXICITY (WITH IRRITATION)

<b>Type:</b>	LD <sub>50</sub>
<b>Value:</b>	> 3.16 g/kg bw
<b>Species:</b>	Rabbit

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<b>Strain:</b>	NZ White
<b>Sex:</b>	Male/Female
<b>Number of Animals:</b>	2 per sex
<b>Vehicle:</b>	None – administered undiluted
<b>Year:</b>	1979
<b>GLP:</b>	Unable to determine
<b>Test Substance:</b>	MRD-79-10 (Raw naphthenic acid derived from kerosene )
<b>Method</b>	<p>3.16 g/kg naphthenic acid was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil.</p> <p>According to experimental protocol, no deaths occurred at the initial level, no addition animals were dosed. If one animal died, the experiment was to be repeated using 3 more groups of animals dosed at varying levels.</p> <p>Following the skin wash, animals were observed for mortality and toxic effects at 2 hr and 4 hr, and once daily thereafter. Body weights were recorded pretest and at termination. Dermal irritation was recorded at 24 hr, 3, 7, 10 and 14 days.</p> <p>The rats were observed 1,2,4, and 6 hours after dosing and once daily for 14 days. Mortality, toxicity and pharmacological effects were recorded. Body weights were recorded pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.</p>
<b>Result:</b>	<p>No deaths occurred at the 3.16 mg/kg dose level. Most of the animals (3/4) appeared normal during the first 2 to 4 hours of dosing, after which symptoms of toxicity were observed. 3 out of 4 animals (1 male, 2 female) showed signs of toxicity until day 12 or 13. During the first 5 days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipisia, anorexia, and few feces.</p> <p>The LD<sub>50</sub> was determined to be greater than 3.16 g/kg bw</p> <p>Redness and irritation scores were recorded at 24 hr, 3, 7, 10 and 14 days post-washing.</p> <p>4 Hour occluded sites (DOT, OECD methods) Mean values (24, 48 &amp; 72 hours) for erythema and edema at the intact sites were 1.69 and 1.3 respectively. The initial response of the skin to the test material was slight, with little difference in response between intact or abraded sites.</p>

The material was judged to be moderately to severely irritating to the occluded skin.

Actual scores were:

**Erythema/Eschar Scores**

Animal Number	1 day	3 day	7 day	10 day	14 day
1M	2	2	4	4	1
2M	1	2	4	4	1
3F	2	4	4	4	0
4F	2	3	4	4	0

Note: All animals showed signs of scar formation after 14 days.

**Edema**

Animal Number	1 day	3 day	7 day	10 day	14 day
1M	3	2	2	2	1
2M	2	3	2	2	0
3F	3	3	2	2	0
4F	3	3	2	2	0

**Reliability:**

(1) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.

**Source**

Exxon, Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, 1979.

**5.2.1 EYE IRRITATION**

**Type:**

EYE IRRITATION

**Species:**

Rabbit

**Strain:**

NZ White

**Sex:**

Male/Female

**Number of Animals:**

3 per sex

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<b>Concentration :</b>	None – administered undiluted
<b>Year:</b>	1979
<b>GLP:</b>	Unable to determine
<b>Test Substance:</b>	MRD-79-10 (Raw naphthenic acid derived from kerosene )
<b>Method</b>	<p>0.1 ml naphthenic acid was placed into the conjunctival sac of eye of each of the six rabbits. The lids were held together briefly to insure adequate distribution. The untreated eye served as a control.</p> <p>The rabbits were observed at 1 and 4 hours, and on days 1, 2, 3, 4, and day 7. If a positive score (any score for iritis or opacity, or a score of 2 or more for redness or chemosis) was noted on day 7, ocular reactions were scored on day 10. Likewise readings on day 14 were given if there was a positive reaction on day 10. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system.</p>
<b>Result:</b>	<p>The following is a summary of data taken from the report: One animal had a positive corneal score that was noted on days 1 and 2. One animal had a positive iris score which was noted during hours 1 and 4. All animals exhibited positive conjunctival scores at some point during the first three days of observation. By day 4, no animals showed positive scores. abraded sites.</p> <p>The material was judged to be an irritant. (According to Draize chart, 4 to 6 rabbits with positive scores observed at 24, 48 or 72 hours). In a later Exxon summary report, eye irritation was judged to be moderate (Exxon, 1980).</p>
<b>Reliability:</b>	(1) Reliable without restrictions; although no indication that it is a GLP study, sufficient detail is provided to make a conclusion about its validity.
<b>Source</b>	Exxon, Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, 1979.

#### 5.4 REPEATED DOSE TOXICITY

<b>Type:</b>	Subchronic (90 Day)
<b>Species:</b>	Rat
<b>Sex:</b>	Females
<b>Strain:</b>	Wistar
<b>Route of administration:</b>	Oral

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<b>Exposure period:</b>	90 days
<b>Frequency of treatment:</b>	1 dose/day (Mon. – Fri, 5 days/week)
<b>Doses/No. of animals:</b>	0.6, 6 or 60 mg/kg bw (aqueous solutions of naphthenic acids); 12 animals per dose level
<b>Control group:</b>	Water – 7.0 ml tap water
<b>Year:</b>	2002
<b>GLP:</b>	Unable to determine
<b>Test Substance:</b>	Naphthenic acid in aqueous solutions (analyzed by mass spectrometry) containing 8549, 845.9 or 84.50 mg/l naphthenic acids derived from Athabasca sands sands tailings.
<b>Method:</b>	<p>Female rats were administered naphthenic acid (orally) at doses of 0.6, 6, or 60 mg/kg/day, 5 days per week for 90 days. Control animals were given 7 ml tap water. All animals were monitored daily . Changes in body weight, food and water consumption and behavioral or clinical signs were recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Similarly, blood samples taken via cardiac puncture on day 91 were analyzed. Following euthanization the liver, kidney, spleen, heart, lung and ovaries were removed, weighed and fixed for microscopic examination.</p> <p>Statistical analysis was performed by using a one-way ANOVA to compare group means for consumption, plasma biochemical/ hematological parameters , and organ weights, while a one-way repeated measure ANOVA was used to compare body weight trends. Probability values of <math>p \leq 0.05</math> was considered statistically significant.</p>
<b>Result:</b>	<p>The following significant effects were seen in the high dose groups:</p> <ul style="list-style-type: none"> <li>• Decreased food consumption immediately following dosing.</li> <li>• Severe, clonic seizures lasting 20 sec (25%) of animals, observed after day 40 – after which all animals, except one that died, resumed normal activity.*</li> <li>• Lower mean body weight throughout the exposure period.</li> <li>• Increased relative organ weights: liver, kidney and brain</li> <li>• Reduction in plasma cholesterol on days 45 and 91 (41 and 43%), Increase in amylase activity on day 45 and 91 (33 and 30%)</li> <li>• Less pronounced differences in total protein concentration (increased) and albumin/globulin ratio (decreased)</li> <li>• 5/12 rats with increased glycogen storage.</li> </ul> <p>The following effects were seen in the mid-dose group:</p> <ul style="list-style-type: none"> <li>• Severe, clonic seizures lasting 20 sec (17%) of animals, observed after day 40 – after which all animals except one that died, resumed normal activity.*</li> <li>• 3/12 rats with increased glycogen accumulation</li> </ul> <p>The following effects were seen in the low-dose group:</p>

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	<ul style="list-style-type: none"> <li>2/12 rats with increased glycogen accumulation</li> </ul>
	<p>*Note: Rats in the low-dose (8%) and control (17%) demonstrated milder episodes, characterized primarily by muscle twitching.</p> <p>Dose-related changes in liver tissue with respect to glycogen accumulation.</p>
<b>Reliability</b>	(2) Reliable with restriction. The study is not a typical subchronic toxicity study as defined by OECD SIDS/HPV, i.e., the study was conducted with female rats only and examined a limited number of organs. However, it is well-conducted and provides limited information regarding potential subchronic effects of naphthenic acids following oral exposure.
<b>Source:</b>	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002a. Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.
<b>Type:</b>	Subchronic (30 Day)
<b>Species:</b>	Mice
<b>Sex:</b>	Male
<b>Strain:</b>	Wistar
<b>Route of administration:</b>	Oral
<b>Exposure period:</b>	30days
<b>Frequency of treatment:</b>	Daily
<b>Doses/No. of animals:</b>	1000 mg/kg bw (no information on number of animals per dose)
<b>Control group:</b>	No information available
<b>Year:</b>	1977
<b>GLP:</b>	Unlikely
<b>Test Substance:</b>	Naphthenic acid – no further information.
<b>Method:</b>	Male rats were given daily oral doses of 1000 mg/kg naphthenic acids. No other experimental details provided in abstract.
<b>Result:</b>	<p>The following statements appeared in the abstract:</p> <p>Repeated daily administration (30 days) of naphthenic acid at doses of 1000 mg/kg orally .. revealed a few cases of (1) CNS depression without analgesia and no loss of the corneal reflex (2) hematological changes, (3) weight loss leading eventually to death due to respiratory arrest, (4) gross morphological changes in the liver and stomach, and (5) histomorphological changes in a few selected organs.</p>
<b>Reliability</b>	(4) Not assignable. This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline

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study, and the level of detail is insufficient to judge its validity.

**Source:**

Pennisi, S., and V.D. Lynch. 1977. Pharmacologist 19: 181. [meeting abstract]

## 5.5 GENETIC TOXICITY IN VITRO

The following salts of naphthenic acid were tested using National Toxicology Program protocols and conducted in accordance with GLP's. Consequently they have ratings of (1), reliable without restriction:

	<u>Calcium Naphthenate</u>	<u>Sodium Naphthenate</u>
<b>Salmonella Mutagenicity Test</b>	Negative	Negative
<b>Chromosome Aberration Test</b>	---	Negative
<b>Sister Chromatid Exchange Test</b>	---	Positive

**Source:** NTP. 2003. <http://ntp-server.niehs.nih.gov/htdocs/Overviews/GenProtocolsPg.html>.

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## 5.6 GENETIC TOXICITY IN VIVO

No data available.

## 5.7 CARCINOGENICITY

<b>Species:</b>	Mice
<b>Sex:</b>	Female
<b>Strain:</b>	No information available
<b>Route of administration:</b>	Dermal
<b>Exposure period:</b>	2 yr
<b>Frequency of treatment:</b>	2 times/day
<b>Doses/No. of animals:</b>	0.05 ml neat - 50 animals
<b>Control group:</b>	No information available
<b>Year:</b>	1987
<b>GLP:</b>	Unknown
<b>Test Substance:</b>	Calcium naphthenate
<b>Method:</b>	Not described; listed in summary as "non-TSCA Protocol/Guideline (voluntary test)"
<b>Result:</b>	<p>The following statements appeared in the abstract:</p> <p>Clinical observations included mild irritation, hair loss, shiny patches on the skin, and flaking skin surfaces. These progressed to moderate irritation (observed with sores and scabs on the treated site), or severe irritation caused by large sores or visible ulcers. In the negative control group, no cutaneous tumors developed at or distant to treated sites. Twelve epidermal and one dermal tumor at the treated sites were observed in eight mice that were exposed to the test material. Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present.</p>
<b>Reliability</b>	(4) Not assignable. This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary.
<b>Source:</b>	U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). <a href="http://www.epa.gov/opptintr/chemtest/naphthst.htm">http://www.epa.gov/opptintr/chemtest/naphthst.htm</a> .

## 5.8 EFFECTS ON REPRODUCTION

<b>Type:</b>	One Generation Reproduction
<b>Species:</b>	Rabbit
<b>Sex:</b>	Male (10)/Female (2)
<b>Strain:</b>	No information available
<b>Route of administration:</b>	Dermal
<b>Frequency of treatment:</b>	6 hr/day, 5 d/wk, 10 weeks
<b>Doses/No. of animals:</b>	2 ml (neat) – 10 male (2 female animals not treated)
<b>Control group:</b>	No information available
<b>Method:</b>	10 week exposure of males prior to mating
<b>Year:</b>	1984
<b>GLP:</b>	Unknown
<b>Test substance:</b>	Calcium naphthenate
<b>Method:</b>	Not described; listed in summary as “non-TSCA Protocol/Guideline (voluntary test)”
<b>Result:</b>	<p>The following statements appeared in the available summary:</p> <p>There were no systemic toxicity, application site toxicity, or statistically significant changes in body weights observed in the test animals during the 10 week exposure period or the 12 week post-exposure period. In the male animals, there were no significant changes in the testes weights. In the females, there were no significant differences in the number of implantations, or in pre-and post-implantation losses. In addition, there were no differences in viable fetuses to those females that were mated with exposed males compared to those mated with unexposed males. The study also reported that there were no macroscopic or microscopic pathological findings in the male reproductive tract.</p>
<b>Reliability:</b>	(4) Not assignable. This information is taken from an EPA site that summarizes results of testing submitted under TSCA. The protocol of the study does not appear to be comparable a guideline study as indicated in the summary.
<b>Source:</b>	U.S. EPA (United States Environmental Protection Agency). 2003. Chemical Information Collection and Data Development (Testing). <a href="http://www.epa.gov/opptintr/chemtest/naphthst.htm">http://www.epa.gov/opptintr/chemtest/naphthst.htm</a> .



## 5.9 DEVELOPMENTAL TOXICITY

<b>Species:</b>	Rat
<b>Sex:</b>	Female
<b>Strain:</b>	Wistar
<b>Route of administration:</b>	Oral
<b>Dose:</b>	0.6, 6 or 60 mg/kg bw
<b>Exposure period:</b>	"Pre-breeding, breeding and gestation" - no other details provided
<b>Frequency of treatment:</b>	Daily
<b>Year:</b>	2002
<b>GLP:</b>	Unknown
<b>Test Substance:</b>	Naphthenic acid isolated from Athabasca oil sands tailings.
<b>Method:</b>	Oral doses of 60 mg/kg/day were given to female rats during pre-breeding, breeding and gestation.
<b>Result:</b>	<p>The following description was given:</p> <p>Reproductive toxicity testing demonstrated dramatic effects on female fertility at an oral dosage of 60 mg/kg/day during pre-breeding, breeding and gestation. While control and low dose (6 mg/kg/day) animals achieved 93 and 100% reproductive success, respectively, only 7% of females dosed at 60 mg/kg/d successfully bore a litter. Total cholesterol of the latter group was 30% lower than controls. Mating and ovulation were comparable amongst control and dose groups, while fetal malformations were not apparent in any offspring. Results suggest that the dose-related infertility may be associated with poor embryonic implantation, an effect that might be secondary to depressed sex hormone production requiring cholesterol as a precursor.</p>
<b>Reliability:</b>	(4) Not assignable. This information is taken from an abstract. The protocol of the study does not appear to be comparable to a guideline study, and the level of detail is insufficient to judge. However, it may be useful in establishing dose levels for a more in-depth study.
<b>Source:</b>	Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002b. Mammalian toxicity of naphthenic acids derived from the Athabasca Oil Sands (AOS). Toxicologist 66(1-S): 64-5. [meeting abstract]